Phytomedicine xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Phytomedicine

journal homepage: www.elsevier.de/phymed

Antileukemia component, dehydroeburicoic acid from *Antrodia camphorata* induces DNA damage and apoptosis *in vitro* and *in vivo* models

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ARTICLE INFO

Keywords:
Antrodia camphorata
Dehydroeburicoic acid (DeEA)
DNA damage
Apoptosis
Topoisomerase II inhibitor

ABSTRACT

Antrodia camphorata (AC) is a native Taiwanese mushroom which is used in Asian folk medicine as a chemopreventive agent. The triterpenoid-rich fraction (FEA) was obtained from the ethanolic extract of AC and characterized by high performance liquid chromatography (HPLC). FEA caused DNA damage in leukemia HL 60 cells which was characterized by phosphorylation of H2A.X and Chk2. It also exhibited apoptotic effect which was correlated to the enhancement of PARP cleavage and to the activation of caspase 3. Five major triterpenoids, antcin K (**1**), antcin C (**2**), zhankuic acid C (**3**), zhankuic acid A (**4**), and dehydroeburicoic acid (**5**) were isolated from FEA. The cytotoxicity of FEA major components (**1**–**5**) was investigated showing that dehydroeburicoic acid (DeEA) was the most potent cytotoxic component. DeEA activated DNA damage and apoptosis biomarkers similar to FEA and also inhibited topoisomerase II. In HL 60 cells xenograft animal model, DeEA treatment resulted in a marked decrease of tumor weight and size without any significant decrease in mice body weights. Taken together, our results provided the first evidence that pure AC component inhibited tumor growth *in vivo* model backing the traditional anticancer use of AC in Asian countries.

Introduction

Antrodia camphorata (AC), also known as *Antrodia cinnamomea* or *Taiwanofungus camphoratus*, is an endemic fungus in Taiwan which only parasitizes in the internal heartwood or the dark humid wood surface of *Cinnamomum kanehirai* (Bull camphor tree) (Ao et al. 2009; Lu et al. 2009a). Mature AC fruiting bodies are regarded as the colored gems of the forests, ruby in Taiwanese forest, due to their colorful orange to brown-red appearance. It takes one year for AC fruiting bodies to grow to the size of one Euro forcing the price of one kilogram to reach 10,000–28,000 €. Due to the slow growth rate and the high price of wild fruiting bodies, AC is widely cultured.

AC, which is known in folk medicine as "chang-chih" has a long history in the prevention and treatment of several life threatening diseases including cancer, inflammatory disorders, and hepatitis (Geethangili and Tzeng 2009; Huang et al. 2010; Lu et al. 2009b; Shen et al. 2003; Yeh et al. 2009). Currently, AC is used as food dietary supplement for cancer prevention and hepatoprotection in several Asian and European countries. Its products are commercially available in China, Japan, Korea, Malaysia, and Singapore. It is also marketed in the United Kingdom under the name *Antrodia camphorata*.

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doi:10.1016/j.phymed.2012.03.014

Our group has been devoted to the anticancer research of AC for many years. In our previous report, we demonstrated that the water extract of AC wild fruiting bodies (ACW) promoted the functional maturation of dendritic cells in the expression of phenotypic characteristics, IL-12 production and chemotaxic activity (Lu et al. 2009b). Moreover, according to ^{1}H NMR analysis, it was suggested that the polysaccharides of ACW could act as a potent adjuvant for cancer immunotherapy through promoting Th1 immune response. We also reported that the ethanolic extract from the wild fruiting bodies of AC (EEAC) could induce HL 60 cell apoptosis and potentiate the anticancer effect of trichostatin A (Lu et al. 2009a). Fractionation of EEAC using different solvents provided three fractions, among them the ethyl acetate fraction (FEA) was the primary contributor to EEAC cytotoxicity. The aforementioned results encouraged us to identify FEA major components and study their cytotoxic activity. We believe that a thorough understanding of the cytotoxic molecular mechanism induced by FEA major components will shed light on the potential utilization of these components as future anticancer agents.

Materials and methods

Assessment of apoptosis

The accumulation of the sub G_1 population in cancer cells was determined by flow cytometry. Cells were seeded onto 6 cm dishes and treated with or without the indicated concentration of the tested samples for 24 h. Cells were then washed twice with PBS and collected by centrifugation at 200 × *g* for 5 min at 4 °C. Cells were fixed in 70% (v/v) ethanol at 4 °C for 30 min. After fixation, cells were treated with 0.2 ml of DNA extraction buffer (0.2 M Na_2HPO_4 and 0.1 M citric acid buffer, pH 7.8) for 30 min, centrifuged, and resuspended in 1 ml of propidium iodide staining buffer (0.1% TritonX-100, 100 μg/ml RNase A, 500 μg/ml of propidium iodide in PBS) at 37 °C for 30 min. Cytometric analyses were performed using a flow cytometer FACS-Calibur (Becton-Dickinson, San Jose, CA, USA) and CellQuest software. Approximately 10,000 cells were counted for each determination.

The externalization of phosphatidylserine (PS) and membrane integrity was quantified using an Annexin V-FLOUS staining kit (Roche Diagnostics GmbH, Mannheim, Germany). In short, 10^6 cells were grown in 35 mm diameter plates and labeled with Annexin V-FLOUS (10 μg/ml) and propidium iodide (PI) (Sigma–Aldrich Corp., St. Louis, MO, USA) (20 μg/ml) prior to harvesting. After labeling, all plates were washed with binding buffer and harvested by scraping. Cells were resuspended in binding buffer at a concentration of 2×10^5 cells/ml before analysis by flow cytometry.

Neutral comet assay for the detection of DNA double-strand breaks (DSBs)

The assay was carried out using a CometAssay™ Kit (Trevigen Inc., Gaithersburg, MD, USA) following the manufacturer's protocol for the neutral comet assay. Briefly, cancer cells (2×10^5 cells/ml) were treated with the tested samples for 18 h. Cells were combined with 1% low melting point agarose at a ratio of 1:10 (v/v). From the mixture, 75 μl were pipetted onto CometSlide™ and allowed to stand at 4 °C in the dark. The slides were immersed in ice-cold lysis solution for 30–60 min. The slides were placed in a horizontal electrophoresis apparatus and electrophoresed in 1× TBE (90 mM Tris–HCl, 90 mM boric acid, and 2 mM EDTA, pH 8.0) at 20 V for 10 min. The samples were then fixed in 70% ethanol and dried before being stained with 1:10,000 SYBR Green I to visualize cellular DNA. The fluorescence images were analyzed using the TriTek Comet Image program to circumscribe the 'head' and the 'tail' regions of each comet and the integrated fluorescence values of each defined area were recorded. The comet length was measured from the trailing edge of the nucleus to the leading edge of the tail. This length was indicative to the extent of DNA damage. Calculations were averaged per replicate.

Western blotting analysis

Cell lysates were prepared as described previously (Yang et al. 2006), by treating the cells for 30 min in RIPA lysis buffer [1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 1 mM sodium orthovanadate, 100 μg/ml phenylmethylsulfonyl fluoride and 30 μg/ml aprotinin, all chemicals were obtained from Sigma–Aldrich Corp.]. The lysates were centrifuged at 20,000 × *g* for 30 min, and the protein concentration in the supernatant was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Equal amounts of proteins were respectively separated by 7.5%, 10% or 12% of SDS-polyacrylamide gel electrophoresis and then electro-transferred to a PVDF membrane. The membrane was blocked with a solution containing 5% fat free dry milk TBST buffer (20 mM Tris–HCl, pH 7.4, 150 mM NaCl and 0.1% Tween 20) for 1 h and washed with TBST buffer. Protein expression was monitored by immunoblotting using specific antibodies: Bcl-2 and Bcl-x (Santa-Cruz Biotechnology, Santa Cruz, CA, USA); caspases 3, 7, 8 and 9; Bax, Bid, Bad, p-H2AX, and PARP (Cell Signaling Technologies, Beverly, MA, USA). Caspases 3, 8, and 9 inhibitors (Z-DEVD-FMK, Z-IETD-FMK, and Z-LEHD-KMK) were purchased from R&D Systems Inc. (Minneapolis, MN, USA). Anti-mouse and rabbit 1gG peroxidase-conjugated secondary antibodies were purchased from Pierce. These proteins were detected by an enhanced chemiluminescence kit (Pierce, Rockford, IL, USA).

Assay of topoisomerase II inhibitors and poisons

The assay was performed as described previously with minor modifications (Giri et al. 2010). Standard relaxation reaction mixtures (20 μl) containing 50 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, 200 mM potassium glutamate, 10 mM dithiothreitol, 50 μg/ml bovine serum albumin, 1 mM ATP, 0.3 μg of pHOT1 plasmid DNA, two units of human topoisomerase II (Topogen, Columbus, OH, USA) and the indicated concentrations of etoposide and DeEA were incubated at 37 °C for 30 min. Reactions were terminated by adding 2 μl of 10% SDS to facilitate trapping the enzyme in a cleavage complex, followed by the addition of 2.5 μl of proteinase K (50 μg/ml) to digest the bound protein (incubated 37 °C for 15 min) and finally by adding 0.1 vol of the sample loading dye. The DNA products were analyzed by electrophoresis through vertical 2% agarose gels at 2 V/cm in 0.5× TAE buffer. Gels were stained with ethidium bromide and photographed using an Eagle Eye II system (Stratagene, La Jolla, CA, USA).

Human leukemia HL-60 cells xenograft animal model

Establishment of nude mice with xenografts was performed as described previously (Dowdy et al. 2006). Six-week-old female immunodeficient athymic mice were purchased from the National Laboratory Animal and Research Center (Taipei, Taiwan). All the animals were maintained under standard laboratory conditions (temperature 24–26 °C, 12–12 h dark–light circle) and fed with laboratory diet and water. The protocols of animal experiments were conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH publication no. 85-23, revised in 1996).

HL 60 cells (1×10^7) resuspended in 0.2 ml PBS were injected s.c. into the right flank of each mouse, and tumor growth was monitored every day. Fourteen days after tumor cell injection, mice with confirmed tumor growth were randomly divided into two groups.

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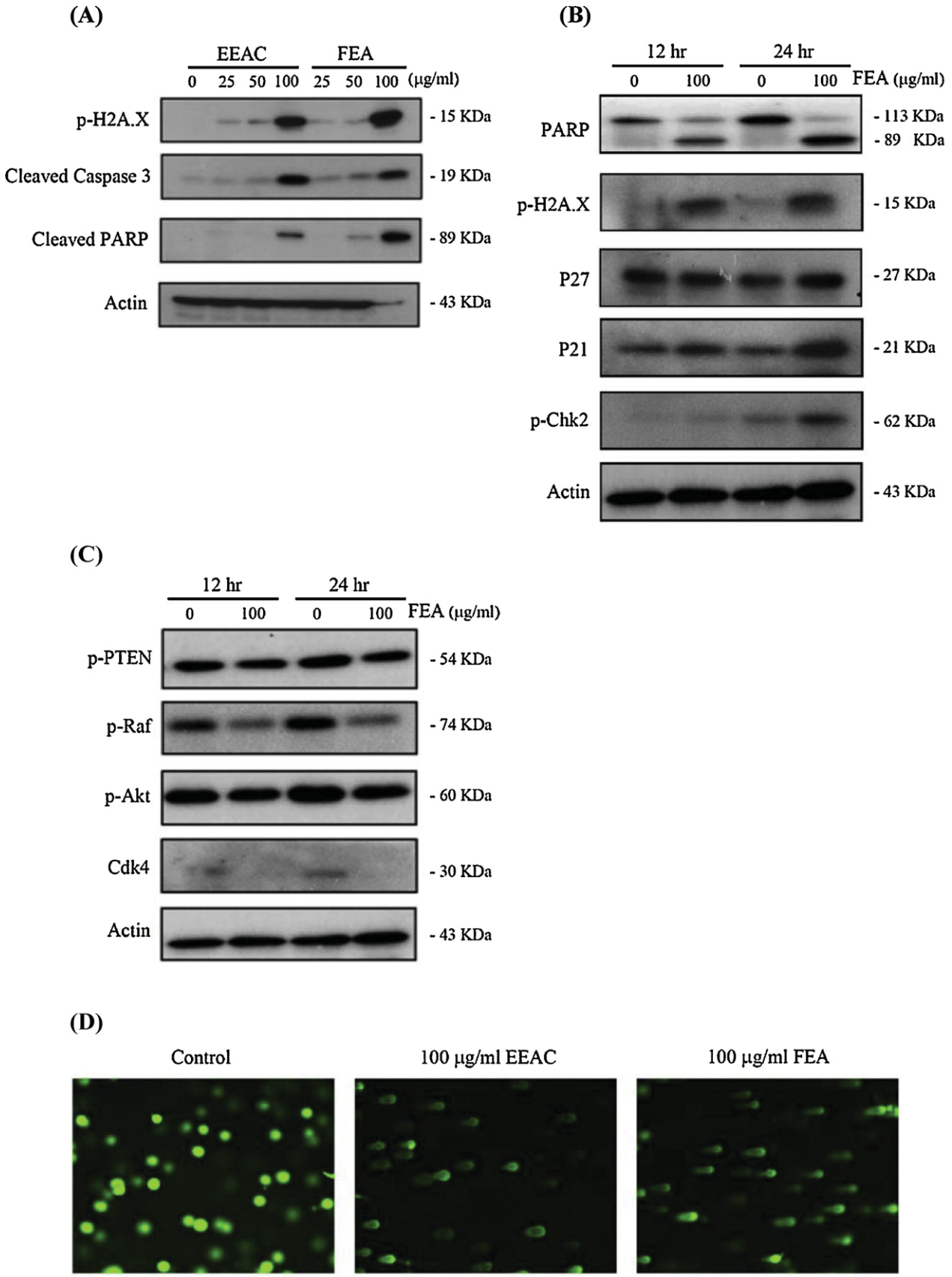

Fig. 1. Western plotting and alkaline comet assays. (A) Effect of EEAC and FEA on apoptotic and DNA damaged-related proteins in HL 60 cells (24 h). (B) Effect of FEA on tumor suppressor genes, apoptotic and DNA damaged-related proteins in HL 60 cells (12 h and 24 h). (C) Effect of FEA on the expression of survival-related proteins and Cdk4 (12 h and 24 h). (D) Assessment of DNA damage by comet assay. HL 60 cells were treated with EEAC or FEA for 18 h and then subjected to the alkaline comet assay to detect the broken DNA (comet tails).

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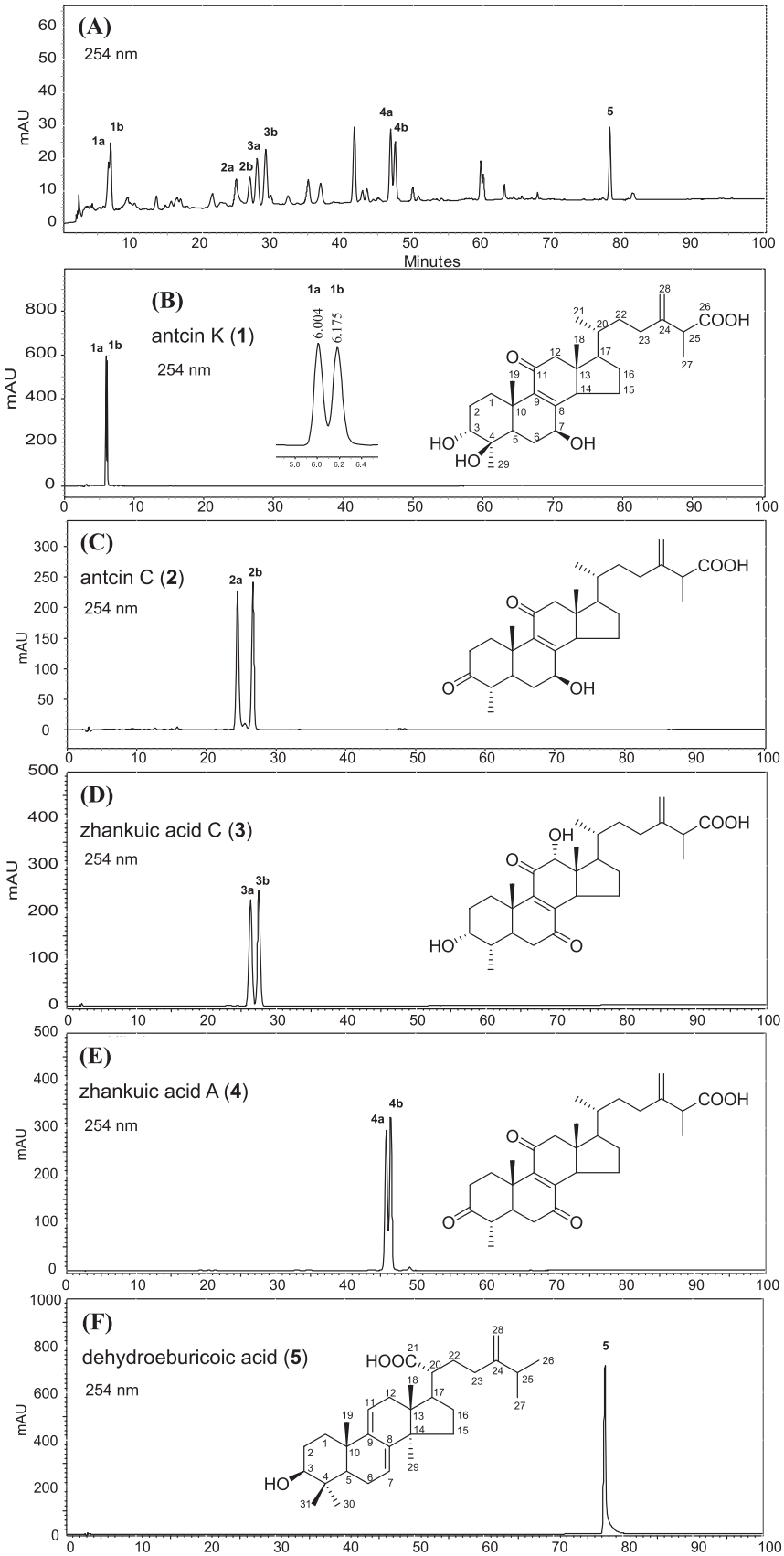

Fig. 2. HPLC chromatogram of the ethyl acetate fraction (FEA, an active triterpenoid-rich fraction) from the ethanolic extract of wild AC. Standard isolates (compounds **1**–**5**) are shown for comparison.

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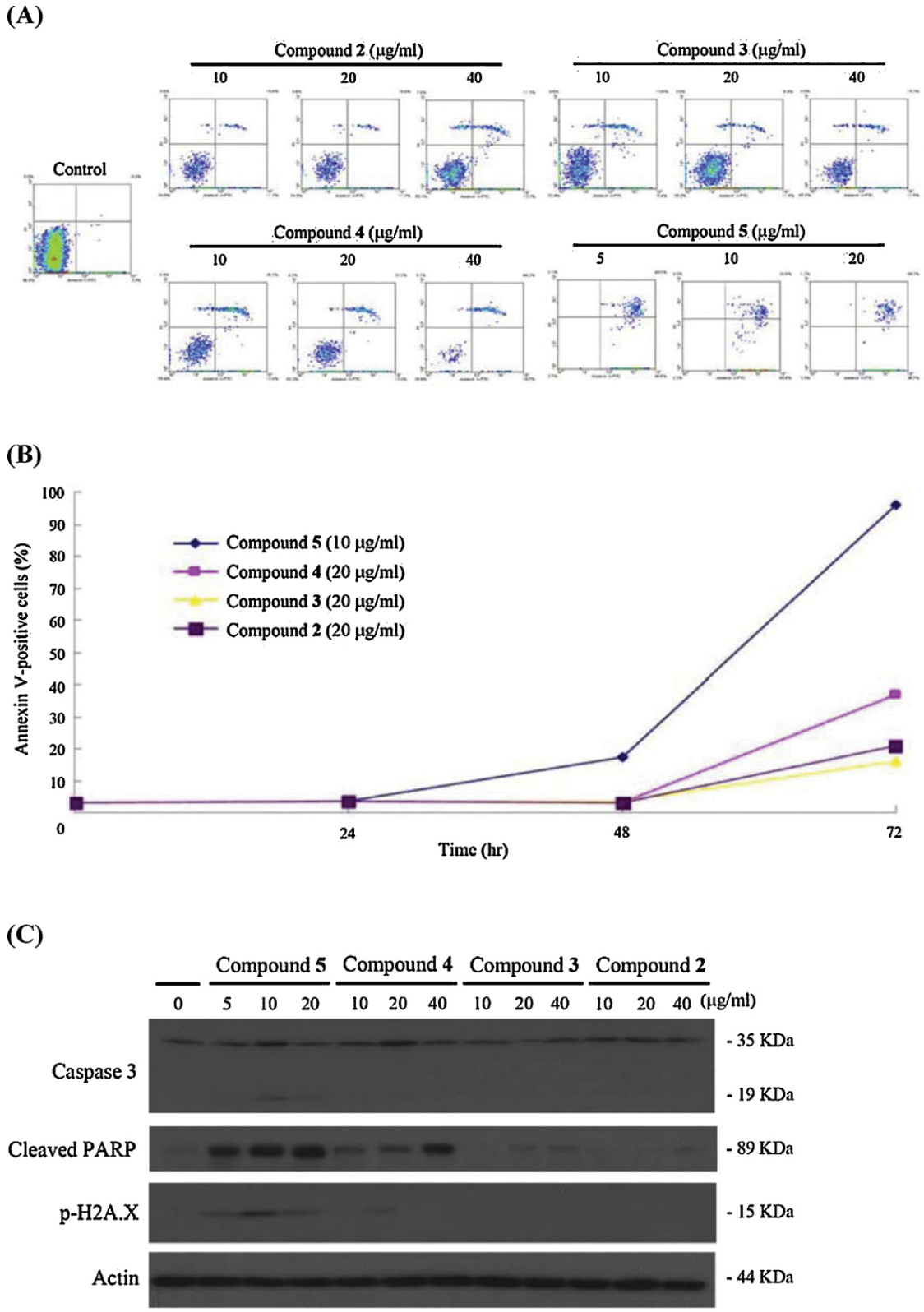

Fig. 3. The effect of FEA compounds **2**–**5** on apoptosis induction and DNA damage in HL 60 cells. (A) FACS dot plots representing HL 60 cells stained with Annexin V and PI after treatment with **2**–**5** (72 h). (B) Effect of **2**–**5** on the percentage of Annexin V-positive cells at different treatment times (24, 48, and 72 h). (C) Western blotting detecting the expression of apoptotic and DNA damaged-related proteins caused by **2**–**5**.

DeEA (10 μg/g) was orally administered to the treatment group and the control group received solvent only. DeEA was administrated every other day for five weeks. Animals were sacrificed by carbon dioxide. The tumors were carefully dissected from each mouse, and tumor weights were measured. Half of the tumor tissue was fixed with formalin and used for immunohistochemical studies, whereas the other half was snap frozen in liquid nitrogen and stored at −80 °C. These tissues were used to extract proteins for

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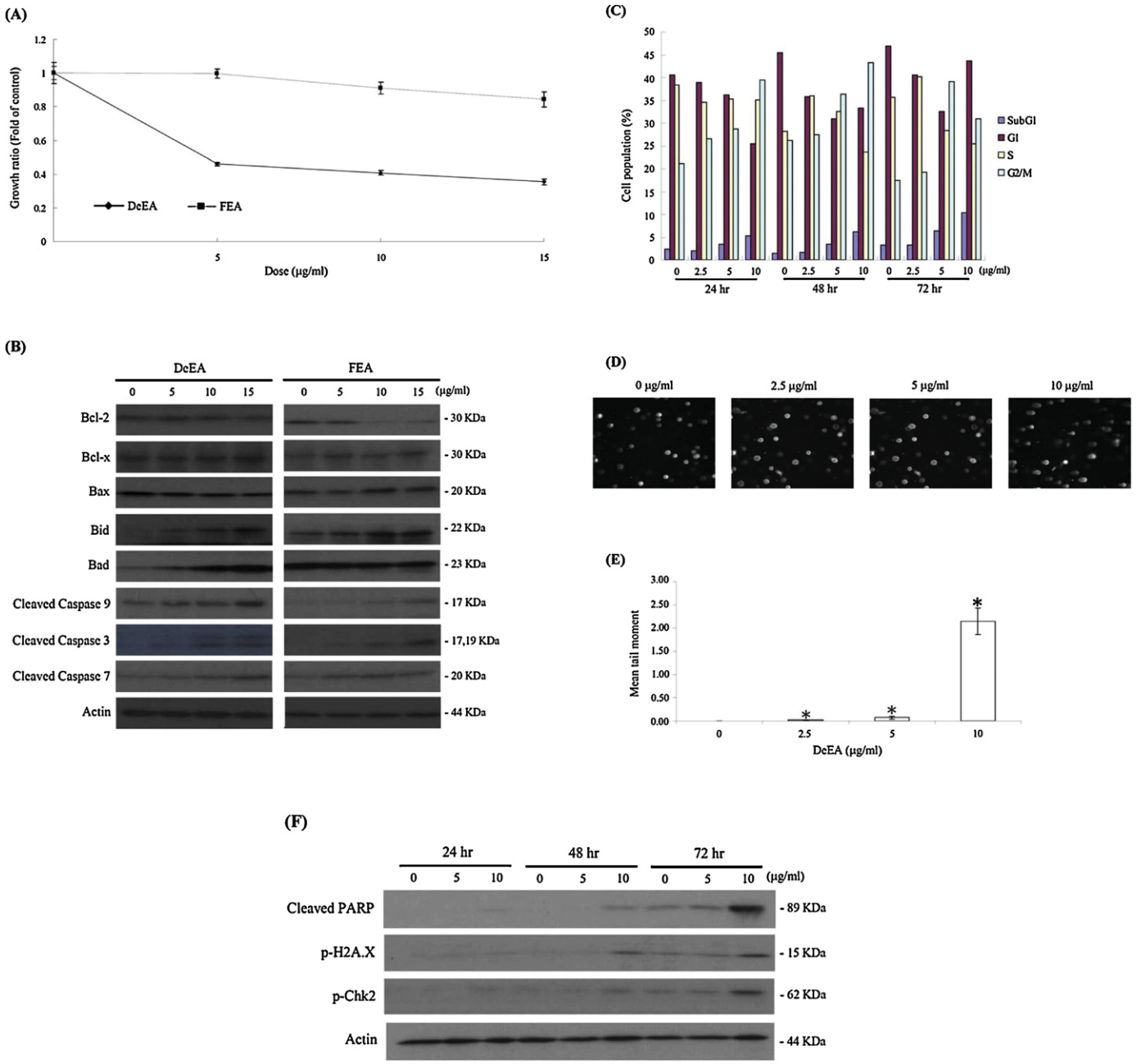

Fig. 4. The cytotoxic effect of DeEA on HL 60 cells. (A) DeEA and FEA cytotoxic activity at various concentrations (72 h) in MTT assay. (B) Effect of DeEA and FEA on anti-apoptotic and pro-apoptotic proteins and caspases 3, 7, and 9 (72 h). (C) Graphical representation of the percentages of cells at SubG$_1$, G$_1$, S and G$_2$/M phases in DeEA-treated cells. (D) Representative images of comet assay showing abnormal comet tails caused by chromosomal DNA double strand breaks in DeEA (2.5, 5 and 10 μg/ml) treated HL 60 cells. (E) DeEA effect on tail movement in HL 60 cells. Histogram was generated by TriTek Comet Image program. Values are expressed as mean ± SEM. *Significantly different from control groups at $p < 0.05$. (F) Effect of DeEA on the cleavage of PARP and on the phosphorylation of H2A.X and Chk2.

western blotting assay. Tumor size was measured three times a week using calipers and tumor volumes were calculated according to the standard formula: width^2 × length/2.

Statistics

The results were expressed as mean ± standard deviation (SD). Comparison in each experiment was performed using an unpaired Student's *t*-test, and a *p* value of less than 0.05 was considered statistically significant.

Results and discussion

The apoptotic and the DNA damaging activities of EEAC and FEA

Our previous study revealed that the ethanolic extract of wild AC (EEAC) and its active fraction (FEA, the ethyl acetate fraction from EEAC) possessed cytotoxicity against leukemia HL 60 cells with IC$_{50}$ values measured after 24 h of 104.82 and 80.53 μg/ml, respectively (Lu et al. 2009a). In the current study, EEAC and FEA were prepared as previously reported (see supplementary data, sections *S2* and *S3*). HL 60 cells were treated for 24 h with 0, 25, 50

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Table 1
Comparisons of peak area and height for the respective components in FEA with HPLC at 254 nm.

Compound	Peak number	Retention time (min)	Area (%)	Height (%)
Antcin K	1a	6.004	4.96	7.08
	1b	6.175	6.23	9.40
Antcin C	2a	24.805	7.34	4.20
	2b	26.733	5.36	4.36
Zhankuic acid C	3a	27.778	6.91	6.59
	3b	28.981	8.07	7.59
Zhankuic acid A	4a	46.825	7.17	9.24
	4b	47.453	6.37	7.68
Dehydroeburicoic acid	5	78.058	4.58	8.79
Others			43.01	35.07
Total			100.00	100.00

and 100 μg/ml of EEAC or FEA and subsequently cell lysates were analyzed by western blotting. FEA or EEAC induced phosphorylation of H2A.X at Ser-139 (γH2A.X) (Fig. 1A) which is considered as a biomarker for DNA double-strand breaks (Kuo and Yang 2008; Mah et al. 2010). Additionally, the use of FEA or EEAC enhanced the cleavage of PARP and the activation of caspase 3, which are both regarded as markers of apoptosis (Fig. 1A). These results implied that FEA is the active apoptotic fraction of EEAC and thus selected for further investigation.

The effect of using FEA (100 μg/ml) on protein expression was also determined. FEA led to a marked increase in the amount of tumor suppressor genes, p21 and p27, enhancement of H2A.X and Chk2 phosphorylation, as well as induction of PARP cleavage (Fig. 1B). On the other hand, phosphorylation levels in each of the survival proteins (Akt, Raf, and PTEN) as well as the expression of Cdk4 decreased following treatment with FEA in a time dependent manner (Fig. 1C). The DNA damaging effect of FEA was further confirmed using alkaline comet assay resulting in comets with abnormal tails sizes (Fig. 1D). The aforementioned results confirmed the DNA damaging and the apoptotic activities of FEA implying its importance as a potential cytotoxic agent (Kim et al. 2011).

The apoptotic and the DNA damaging activities of FEA major components

FEA major components were separated by normal phase chromatography and purified by reverse phase high performance liquid chromatography (HPLC) yielding five major constituents (see supplementary data, section *S4*). The isolated compounds were four ergostanes, antcin K (**1**), antcin C (**2**), zhankuic acid C (**3**), and zhankuic acid A (**4**) as well as one lanostane, dehydroeburicoic acid (**5**) (Chen et al. 1995; Shen et al. 2003). The isolation of these major triterpenes as standards led to the establishment of HPLC fingerprint of FEA (Fig. 2) (see supplementary data for detailed HPLC condition, section *S5*). According to the published report (Chen et al. 1995), each AC ergostane triterpene (compounds **1**–**4**) exists as a pair of stereo isomers due to the presence of chiral center at C-25. Two peaks representing an isomeric pair of each ergostane can be observed in the HPLC fingerprint. For example, peak pairs 1a and 1b, 2a and 2b, 3a and 3b, as well as 4a and 4b corresponding to antcin K (**1**), antcin C (**2**), zhankuic acid C (**3**), and zhankuic acid A (**4**), respectively were observed (Fig. 2B–E). On the other hand, dehydroeburicoic acid with no chiral center at C-25 existed only as a single isomer, peak 5 (Fig. 2F). Peak areas and heights of FEA major components (**1**–**5**) at 254 nm were calculated and compared as shown in Table 1. The areas of the labeled peaks (**1**–**5**) accounted for 56.99% of the total peak areas observed in the HPLC fingerprint.

Regarding the antiproliferative activity of **1**–**5** against leukemia HL 60 cells, **1** showed no activity even at a high concentration (100 μg/ml). Compounds **2**–**5** exhibited antiproliferative activity against HL 60 cells and were further investigated using Annexin V and PI double staining technique. HL 60 cells were treated by different concentrations of antcin C (**2**), zhankuic acid C (**3**), zhankuic acid A (**4**), and dehydroeburicoic acid (**5**) for 24, 48, and 72 h. Cells were stained with Annexin V/PI and examined with flow cytometry. FACS dot plots representing Annexin V and PI staining after the treatment with **2**–**5** are shown in Fig. 3A. The quantification of Annexin V/PI experiment revealed that **4** and **5** increased the apoptotic population of HL 60 cells in a dose dependent manner. On the other hand, only **5** (10 μg/ml) induced apoptosis in a time dependent manner (Fig. 3B).

Compounds **4** and **5** induced cleavage of PARP in a dose dependent manner (Fig. 3C). The treatment of HL 60 cells with **4** (20 μg/ml) and **5** (10 μg/ml) induced the activation of caspase 3 and the phosphorylation of H2A.X. The analytical and the biological assays clearly implied that **5** (DeEA) is FEA major component with the most potent apoptotic and DNA damaging activities.

The apoptotic and the DNA damaging activities of DeEA

To further confirm the fact that DeEA is the lead active compound of FEA (triterpenoid-rich fraction) the cytotoxicity of DeEA and FEA against HL 60 cells was compared (Fig. 4A). DeEA exhibited more potent cytotoxicity compared to FEA with IC_{50} 3.39 and 22.4 μg/ml, respectively, after 72 h as indicated by MTT assay. The effect of DeEA and FEA on the expression of anti-apoptotic (Bcl-2 and Bcl-x) and pro-apoptotic (Bax, Bid, and Bad) proteins as well as the activation of caspases 3, 7, and 9 was also investigated (Fig. 4B). DeEA was more potent compared to FEA in enhancing the expression of pro-apoptotic proteins Bid and Bad. Also DeEA induced the activation of caspases 3, 7, and 9 and similar results were observed upon the treatment of FEA. To analyze whether the apoptotic induction of DeEA and FEA involves intrinsic or extrinsic apoptotic pathways, specific inhibitors for caspases 3, 8, and 9 were tested. Pretreatment of cells with caspase inhibitors did not inhibit the apoptotic effect of DeEA and FEA, suggesting that the apoptotic effect of DeEA and FEA is independent on caspase activation (data not shown).

Treatment of HL 60 cells with increasing doses of DeEA (0, 2.5, 5 and 10 μg/ml) resulted in G2/M distribution rates of 21.1%, 26.6%, 28.7% and 39.5%, respectively after 24 h (Fig. 4**C**). Similar results were observed after 48 and 72 h. Thus DeEA treatment appeared to induce G2/M phase arrest in a dose dependent manner.

To clarify the role of DeEA as DNA damaging agent, we treated HL 60 cells with various concentrations of DeEA for 18 h and analyzed

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the level of tail movement with the alkaline comet assay. DeEA at 10 μg/ml caused severe DNA damage in HL 60 cells as indicated by abnormal tail size in comet assay (Fig. 4D and E).

To examine the extent of DNA damage which can lead to the activation of cell cycle checkpoints in HL 60 cells, the effect of DeEA on PARP, p-H2A.X and p-Chk2 was studied (Fig. 4F). DeEA at 10 μg/ml significantly increased the phosphorylation of Chk2 and H2A.X, but did not change the phosphorylation of Chk1 (data not shown). Additionally, DeEA induced PARP cleavage. These findings confirmed the apoptotic and the DNA damaging activities of DeEA on HL 60 cells.

The inhibitory activity of DeEA on topoisomerase II

Topoisomerases are essential enzymes for DNA metabolism and their inhibitors are emerging as potential cytotoxic agents. Exposure of cells to DNA topoisomerase I (topo I) or topoisomerase II (topo II) inhibitors leads to DNA damage that often involves

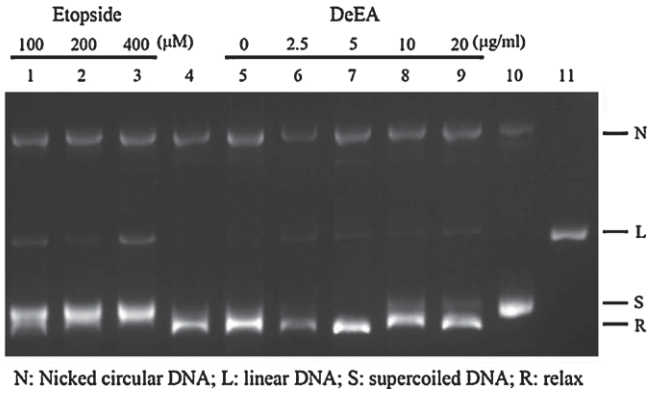

Fig. 5. Effect of DeEA on topoisomerase II mediated supercoiled pHOT1 plasmid DNA relaxation. Lane 1–3: positive control, etoposide (100, 200 and 400 μM); Lane 4: negative control plasmid DNA; Lane 5–9: DeEA (0, 2.5, 5, 10, and 20 μg/ml); Lane 10: plasmid DNA + topoisomerase II; Lane 11: linear DNA.

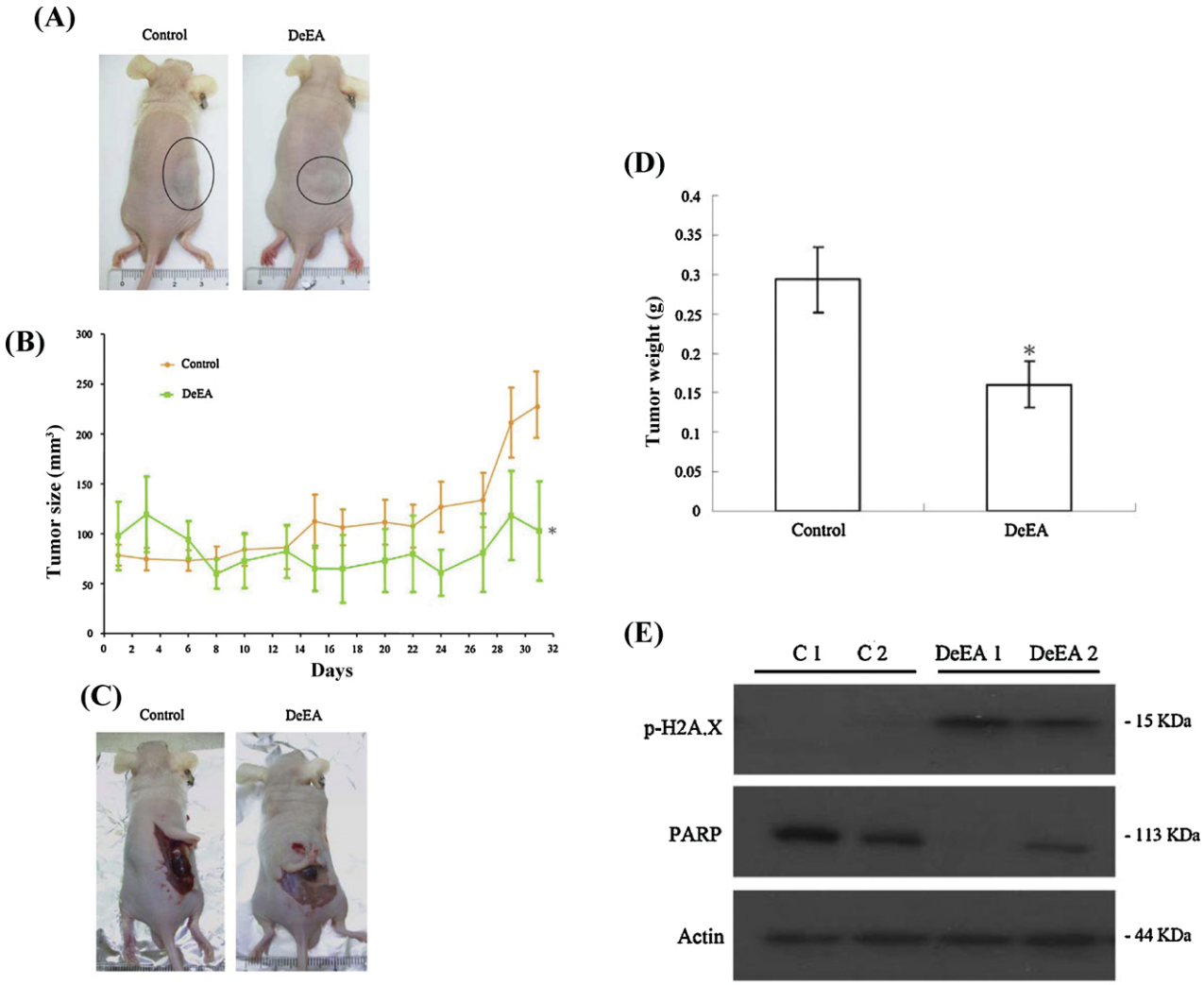

Fig. 6. Effect of DeEA on HL 60 cells tumor growth in xenograft animal model. Female nude mice bearing leukemia HL 60 tumors were treated with the solvent (negative control) or DeEA (10 μg/g) for 5 weeks. (A) A representative picture of tumor growth in xenograft nude mice administrated solvent only (left) and DeEA (right). (B) Tumor volumes were measured every other day, and results are expressed as mean ± SEM. *Significantly different from control groups at $p < 0.05$. (C) A representative picture of tumor tissue as it appears in the right flank of the mouse. (D) Histogram of the tumor weight from the control group and DeEA treated group. Values are expressed as mean ± SEM. *Significantly different from control groups at $p < 0.05$. (E) Effect of DeEA on PARP and on H2A.X in tumor samples. Two mice were picked randomly from each group. C1 and C2 represent two mice from the control group. DeEA1 and DeEA2 represent two mice from the DeEA treated group chosen randomly.

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the formation of DNA double-strand breaks (Darzynkiewicz et al. 2009; Deng et al. 2009). Mizushina et al. (2004) revealed that dehydroebriconic acid, from the sclerotium of *Poris cocos* inhibited DNA topo II with no activity on DNA topo I. The structure of dehydroebriconic acid is similar to DeEA except the hydroxyl group at C-3 in DeEA is replaced by a carbonyl group in dehydroebriconic acid.

The inhibitory activity of dehydroebriconic acid on DNA topo II encouraged us to examine whether DeEA treatment can induce DNA damage in leukemia cells through inhibition of topo II or not. For this purpose, a cell-free DNA cleavage assay was performed using enzyme-mediated negatively supercoiled pHOT1 plasmid DNA. It was found that DeEA induced DNA cleavage in the presence of topo II (Lanes 6–9) (Fig. 5), as indicated by the appearance of linear DNA (marked by L). The results were similar to those obtained upon utilizing etoposide, a standard topo II inhibitor (Lanes 1–3) (Das et al. 2007).

The inhibitory effect of DeEA on tumor growth in xenograft animal model

HL 60 cells were inoculated subcutaneously at the right flank of female immunodeficient athymic mice. After 5 weeks of treatment, the tumor growth of HL 60 cells was significantly suppressed by oral administration of DeEA (10 μg/g) (Fig. 6A). The average tumor size on day 31 in the control group was 229.46 mm^3, whereas the average tumor size in the DeEA-treated group was 102.75 mm^3 (Fig. 6B). The tumor size was significantly lower in the DeEA-treated group as compared to the control group ($p < 0.05$) with no significant difference in the mice body weights. At the end of the treatment, the tumor tissue was isolated and weighed (Fig. 6C). The mean of tumor weights was obviously less in the DeEA-treated group compared to the control group ($p < 0.05$) (Fig. 6D). The western blotting analysis of tumor tissue indicated that the level of PARP was decreased and the phosphorylation of H2A.X was increased in DeEA-treated group compared to the control group (Fig. 6E).

Conclusion

In this study, we illustrated the apoptotic and the DNA damaging activities of the triterpenoid-rich fraction (FEA) and its major components obtained from the ethanolic extract of *A. camphorata*. FEA induced PARP cleavage and caspase 3 activation indicating its apoptotic activity. It also induced the phosphorylation of H2A.X as a marker of DNA double strand break. From the active FEA fraction, dehydroeburicoic acid (DeEA) was isolated as the lead most active component. DeEA induced G2/M phase arrest in a dose dependent manner in HL 60 cells. It also induced apoptotic and DNA damaging markers revealing its role as the potent cytotoxic constituent of FEA. Additionally it inhibited topo II suggesting the possible role of this enzyme in the cytotoxic activity of DeEA. The antitumor effect of DeEA was further demonstrated in reducing tumor weight and size in xenograft animal model. The present findings not only support the ethnopharmacological use of *A. camphorata* but also divulge the antitumor activity of dehydroeburicoic acid as the major lead compound of this treasured mushroom.

Conflict of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by the National Science Council grants (NSC 98-2324-B-037-001 and 98-2321-B-037-060) and the Department of Health, Executive Yuan, Taiwan (DOH99-TD-C-111-002). This work was also supported by the Academic-Industrial Collaborative Project of Kaohsiung Medical University and Yung Peng Biotechnology Co., Ltd., Kaohsiung (S099009 and 100A502).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2012.03.014.

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Please cite this article in press as: Du, Y.-C., et al., Antileukemia component, dehydroeburicoic acid from *Antrodia camphorata* induces DNA damage and apoptosis *in vitro* and *in vivo* models. Phytomedicine (2012), doi:10.1016/j.phymed.2012.03.014