Antitumor Activity of Triterpenoid Saponin-Rich *Adisia gigantifolia* Extract on Human Breast Adenocarcinoma Cells *in Vitro* and *in Vivo*

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The aim of this study was to explore whether the ethanolic extract of *Adisia gigantifolia* rhizomes (AGB-5), a traditional herbal medicine from China, could affect the proliferation of human breast adenocarcinoma (MCF-7) cells *in vitro* and to explore the antitumor effects of AGB-5 in BALB/c mice engrafted with MCF-7 cells. The results showed that AGB-5 markedly inhibited the proliferation of MCF-7 cells with an IC₅₀ value of 11.89±1.12 µg/mL, increased the S phase and decreased the G2/M phase without influence on G1 phase. MCF-7 cells treated with AGB-5 presented a dose-dependent increase of apoptosis compared with the control group. AGB-5 also significantly increased the activity of caspase-3 and -9 in a dose-dependent manner in MCF-7 cells. Furthermore, in an *in vivo* model, AGB-5 reduced tumor volume, brought back the red blood cell (RBC) and white blood cell (WBC) count near to normal value, enhanced superoxide dismutase and catalase level of MCF-7 bearing mice. This is the first study to verify the antitumor activity of *A. gigantifolia* *in vivo*. The results suggest that AGB-5 may have potential beneficial effects against human breast adenocarcinoma.

Key words anticancer; cell cycle arrest; apoptosis; catalase; superoxide dismutase (SOD)

Many of the currently used cancer chemotherapy drugs are either directly derived or chemically synthesized active principles of natural products.¹⁾ Natural products have proven to be rich sources of leads for compounds with anticancer properties. According to a recent report, 63% of anticancer agents approved between 1981 and 2008 were natural products, natural-product-derived, or natural product-inspired.²⁾ The discovery of novel anticancer agents has progressed considerably in recent years. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials all have demonstrated that saponins have significant potential in cancer chemoprevention and chemotherapy.^{3–7)}

The dried rhizome of *Adisia gigantifolia* STAPF. (Myrsinaceae) is mainly used as Chinese folk medicine in south of China for the treatment of rheumatism, muscle and bone pain, and traumatic injury. A kind of evergreen dwarf shrub, it is distributed in the provinces of Guangxi, Jiangxi, and Fujian in China.⁸⁾ Previous chemical studies showed that triterpenoid saponins were the main components from this genus. Many triterpenoid saponins have been isolated from the rhizome of *A. gigantifolia*.⁹⁾ We recently isolated a series of new oleanane-type triterpenoid saponins from the rhizome of *A. gigantifolia* with the former compounds exhibiting cytotoxicity against human cancer cell lines.^{10–12)}

The current study is one of the first efforts to verify the antitumor activity of *A. gigantifolia* using an animal model. Ethanol extract of *A. gigantifolia* rhizome (AGB-5) was selected for this study because it is rich in active saponins. The present study was designed to explore the mechanisms of the antitumor effects of AGB-5 on the MCF-7 cell line, as well as the antitumor effects of AGB-5 in BALB/c mice injected with MCF-7 cells.

MATERIALS AND METHODS

Reagents and Materials Human breast adenocarcinoma cell line (MCF-7) was obtained from Chinese Academy of Medical Sciences tumor cell bank, Beijing, China. All the chemicals were of analytical grade. Ethylene diamine tetraacetic acid (EDTA), pyrogallol, hydrogen peroxide, sodium dihydrogen orthophosphate, potassium dihydrogen orthophosphate, ferric chloride, hematoxylin, were obtained from Sinopharm Chemical Reagent Limited Corporation (Beijing, China). HPLC-grade methanol (Merck, Darmstadt, Germany) was utilized for the HPLC analysis.

Plant Material The rhizome of *A. gigantifolia* was obtained from Guangdong, China in 2007 and was authenticated by Prof. Ping Liu of PLA Traditional Chinese Medicine Pharmacy. The voucher specimen (collection No. 029) has been deposited in traditional PLA General Hospital, Beijing, China. The rhizomes were dried in shade, powdered, and stored in an air-tight container for further use.

Preparation and Analysis of AGB-5 The dried and powdered rhizome parts of *A. gigantifolia* (13.5 kg) were percolated with 60% EtOH (38.5 L). The 60% EtOH extract (1.54 kg) was suspended in H₂O and partitioned between petroleum (8 L×2) and water, the petroleum was removed. Then, the water portion was partitioned with ethyl acetate (10 L×2) and the ethyl acetate was also removed. Finally, the water portion was partitioned with *n*-butanol (10 L×3). After removing the solvent, the *n*-butanol layer (600 g) was subjected to a macroporous resin D101 column (5 kg, 11×82.5 cm) and eluted with H₂O (16 L) and 30% EtOH (68 L), 50% EtOH (30 L), 70% EtOH (32 L), and 95% EtOH (20 L) in turn. The 50% EtOH eluate (30 L) was concentrated to get the crude extract (AGB-5, 50 g, 0.37%, w/w), which was kept in a desiccator prior to use for the *in vitro* and *in vivo* studies.

The main constituents of AGB-5 are saponins and HPLC

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(Hitachi, Japan) was utilized to quantify the accurate amount of saponins 1–5 using evaporative light scattering (ELSD) detector (Alltech, America). The HPLC system was composed of L-2200 autosampler (Hitachi, Japan), L-2130 pump (Hitachi, Japan), ELSD 2000ES detector (Alltech, America) and Agilent HC C18 (4.6×250mm) column. A gradient solvent system was 55% CH₃OH–90% CH₃OH, (0–40min), flow rate was 0.8 mL·min⁻¹. The temperature of drift tube was 100°C and the air flow rate was 2.8 L·min⁻¹. The amount of saponins 1–5 in AGB-5 were determined to be 61.33, 3.47, 3.09, 4.25 and 2.85% (Fig. 1A). The reference compounds cyclamiretin A 3 β -O- α -L-rhamnopyranosyl-(1→3)-[β -D-xylopyranosyl-(1→2)]- β -D-glucopyranosyl-(1→4)-[β -D-glucopyranosyl-(1→2)]- α -L-arabinopyranosyl (1), cyclamiretin A 3 β -O- α -L-rhamnopyranosyl-(1→3)-[β -D-xylopyranosyl-(1→2)]- β -D-glucopyranosyl-(1→4)-[β -D-6-O-acetylglucopyranosyl-(1→2)]- α -L-arabinopyranosyl (2), 3 β -O- α -L-rhamnopyranosyl-(1→3)-[β -D-xylopyranosyl-(1→2)]- β -D-glucopyranosyl-(1→4)-[β -D-glucopyranosyl-(1→2)]- α -L-arabinopyranoside-16 α -hydroxy-13,28-epoxy-oleanane (3), 3 β -O- α -L-rhamnopyranosyl-(1→3)-[β -D-xylopyranosyl-(1→2)]- β -D-glucopyranosyl-(1→4)-[β -D-glucopyranosyl-(1→2)]- α -L-arabinopyranosyl-16-oxo-13 β ,28-epoxy-oleanane (4) and 3 β -O- α -L-rhamnopyranosyl-(1→3)-[β -D-glucopyranosyl-(1→4)]- β -D-xylopyranosyl-(1→2)]- β -D-glucopyranosyl-(1→4)-[β -D-glucopyranosyl-(1→2)]- α -L-arabinopyranosyl-16 α -hydroxy-13 β ,28-epoxy-30-acetoxyleane (5) were obtained from *A. gigantifolia* with >95% purity, the structures of compounds 1–5 are shown in Fig. 1B.

Animals BALB/c-nu mice of either sex weighing 18–22 g were obtained from the experimental animal center of the Academy of Military Medical Sciences, Beijing, China. They were acclimatized for one week before the experiment and maintained under controlled conditions (temperature, 25±2°C along with a light and dark cycle of 12h each) and were fed a standard pellet diet along with water *ad libitum*. All animal experiments were conducted in accordance with the internationally accepted principles for laboratory animal use and care as described in the European Community guidelines.¹³⁾ and were approved by the Animal Ethics Committee of our hospital (09182).

Assay for Cells Growth Inhibition Cells were seeded in 96-well plates at a density of 0.8×10⁴ cells/well and incubated for 24h. Test samples were dissolved in dimethyl sulfoxide (DMSO) and added to the medium. Following a 48h incubation, the wells were incubated with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (100 μ L/well concentrated at 5 mg/mL) at 37°C for 4h. The supernatant was aspired, and the 200 μ L of DMSO was added to redissolve the formazan crystals. The optical density was measured by an enzyme-linked immunosorbent assay (ELISA) plate reader at 490 nm (Perkin-Elmer, Inc., 1420-012, China). The antiproliferative effects of the extract and cisplatin (DDP, sigma, >99%) was determined at 0, 5, 10, 20 and 40 μ g/mL (each concentration was tested in quintuple wells), respectively. Data were calculated as percentage of inhibition by the formula: % inhibition=[100-(OD_i/OD_s)×100]%, OD_i and OD_s being the mean optical densities of the test compounds and the solvent control, respectively. The experiments were repeated three times and antiproliferative effects were expressed as the IC₅₀ value, which reduces the number of viable cells by 50%.

Cell Cycle Distribution Assay Cell cycle distribution assay was conducted using the cell cycle detection kit (KeyGen Biotech. Co., Ltd., Nanjing, China) following the manufacturer's instruction. Cells were subjected to flow cytometric analysis using FACS Calibur flow cytometer (BD Biosciences, U.S.A.). Events were evaluated for each sample and the cell cycle distribution was analyzed using ModFit LT for Mac v 3.0 (BD Biosciences).

Apoptosis Analysis Apoptosis was detected by Annexin V-fluorescein isothiocyanate (FITC) and PI double staining. MCF-7 cells were treated with various concentrations of AGB-5 or 0.1% DMSO for 24h, then collected and stained using Annexin V-FITC/PI staining kit (KeyGen Biotech. Co., Ltd.) according to manufacturer's instruction. Briefly, cells were harvested, washed with phosphate buffered saline (PBS) and re-suspended in 500 μ L PBS plus Annexin V-FITC and PI. Percentages of apoptosis cells were determined by flow cytometry (FACSCalibur; Becton Dickinson, San Jose, CA, U.S.A.).

Apoptosis Assessment by Hoechst 33258 Staining Apoptotic morphological changes were observed by Hoechst 33258 staining. MCF-7 cells were seeded in 6-well plates at a concentration of 2×10⁵ cells/well and treated with AGB-5 (0, 5, 10 μ g/mL) for 24h. The attached cells were washed twice with PBS and fixed with 4% formaldehyde for 20min, then stained with Hoechst 33258 (Beyotime, Haimen, China) for 10min. After being washed with PBS, the stained cells were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

Caspase-3 and -9 Enzyme Assay The activities of caspase-3 and -9 protein were determined following the test kit instructions (RandD Systems, Inc., Minneapolis, U.S.A.). Briefly, MCF-7 cells treated with AGB-5 for 24h were collected, washed twice with PBS and centrifuged at 500×g for 5min. The cell pellet was suspended in lysis buffer and allowed to swell on ice for 15min before being centrifuged at 18000×g for 15min. The supernatant (10 μ L), reaction buffer (80 μ L) and caspase-3, and -9 substrate (DEVD-pNA, LEHD-pNA) were added. After incubating at 37°C for 1–2h in darkness, the absorbance was measured at 405nm, with the lysis buffer (10 μ L) and reaction buffer (90 μ L) as control.

Acute Toxicity Mice were intraperitoneally injected with AGB-5 at various doses (10, 40, 80, 100 mg/kg/d) respectively. One group was maintained as control and was given distilled water. They were observed for toxic symptoms continuously for 2h. Finally, the number of survivors was noted after 24h. In the toxicity study, no mortality occurred within 24h under the tested doses of AGB-5.

In Vivo Antitumor Study BALB/c-nu mice were divided into 6 groups (*n*=6). Antitumor activity of the extract was measured by slightly modifying the method described by Gupta *et al.*¹⁴⁾ Briefly, all the groups were injected with MCF-7 cells (0.2mL of 2×10⁶ cells/mouse) subcutaneously into the right anterior armpit except the normal group (group 1). This was considered as day zero. After 7d of tumor inoculation, saline (5mL/kg/mouse/d) were injected to normal (group 1) and MCF-7 control (group 2) groups respectively for 7d *in situ* administration. Similarly, AGB-5 at different doses 3, 6, and 12mg/kg/mouse/d in saline and standard drug DDP (2mg/kg/mouse/d) were *in situ* administered in groups 3, 4, and 5, respectively. After the administration of the last dose followed by 18h fasting, mice from all groups were sacrificed

A

B

Fig. 1. (A) HPLC Chromatogram of AGB-5 and (B) Chemical Structures of Main Saponins in AGB-5

to determine the antitumor activity of AGB-5 and to evaluate its effect on hematological and liver related biochemical parameters.

The antitumor effect of AGB-5 was assessed by observation of changes with respect to body weight and tumor volume. Hemoglobin content, red blood cell (RBC) and white blood cell (WBC) counts were measured from orbital venous plexus blood.

After the collection of blood samples, the mice were sacrificed and their livers were excised, rinsed in ice cold normal saline followed by cold 0.15 mol/L Tris-HCl buffer (pH 7.4), blotted dry, and weighed. A 10% w/v homogenate was prepared in 0.15 mol/L Tris-HCl buffer (pH 7.4) and a portion utilized for the estimation of lipid peroxidation. The remain-

ing homogenate was centrifuged at 1500rpm for 15min at 4°C. The supernatant obtained was used for the estimation of superoxide dismutase and catalase.

Determination of Superoxide Dismutase (SOD) and Catalase Activities For the biochemical analyses of these enzymes, livers of MCF-7-bearing mice were homogenized in phosphate buffer (pH 6.5). SOD activity was measured in both tissues by the ability of this enzyme to inhibit pyrogallol auto-oxidation using a spectrophotometer at 420 nm.¹⁵ The amount of enzyme that inhibited the reaction by 50% (IC₅₀) was defined as one unit of SOD, and the enzyme activity was expressed in units of SOD per mg of protein. Catalase (CAT) activity was measured according to Takahara *et al.*¹⁶ The reaction was monitored at 240 nm in spectrophotometer for liver

samples to detect H_2O_2 degradation per minute.

Statistical Analysis All results about the screening test were expressed as mean $\pm$ standard error (S.E.M.), respectively. ANOVA was used to analyze the statistical significance of the results. A probability value of $p < 0.05$ was considered to be significant. All calculations were performed using: SPSS, version 11.5.

RESULTS

In Vitro Cytostatic Effects Assay The ethanol extract of *A. gigantifolia* rhizome (AGB-5) showed marked cytotoxic activity to the MCF-7 cell lines in a dose-dependent manner with IC_{50} value of $11.89 \pm 1.12 \mu\text{g/mL}$, whereas the IC_{50} of the positive control DDP was $13.31 \pm 2.17 \mu\text{g/mL}$ (Fig. 2).

As shown in Fig. 3, the percentage of cells in G2/M and S phase was 22.93% and 24.26%, respectively, in control. As

Fig. 2. Dose-Response Curves of the Antiproliferative Effects of AGB-5 and DDP on MCF-7 Cells

Cells were seeded in 96-well plates at a density of 0.8×10^4 cells/well and then treated with 0, 5, 10, 20 and $40 \mu\text{g/mL}$ of AGB-5 and DDP for 48 h, each concentration was tested in quintuple wells.

compared to controls, the cells treated with AGB-5 ($10 \mu\text{g/mL}$) significantly increased in the S phase and decreased in the G2/M phase ($p < 0.01$). AGB-5 showed no significant influence on proportion of cells in G1 phase, which inferred that AGB-5 arrested MCF-7 cells in G2/M and S phases.

As shown in Fig. 4A, MCF-7 cells treated with AGB-5 presented a dose-dependent increase of apoptosis compared with the control group. Quantitative results showed that the percentage of Annexin V-positive cells was $6.85 \pm 0.51\%$, $11.31 \pm 0.96\%$ and $26.52 \pm 1.68\%$ in MCF-7 cells treated with AGB-5 (0, 5, $10 \mu\text{g/mL}$). Further, apoptosis was confirmed by examining the nuclear morphology with Hoechst 33258 staining. As shown in Fig. 4B, MCF-7 cells not treated with AGB-5 were normally blue. It was worth noting that, for AGB-5 treatment, the cells displayed strong blue fluorescence and indicated typical apoptotic morphology after 24 h. The observations revealed that AGB-5 induced apoptosis against MCF-7 cell line.

The enzyme activities of caspase-3 and caspase-9 were determined by spectrophotometry. The results in Fig. 4 showed that caspase-3 and -9 were activated in MCF-7 cells after 24 h of AGB-5 ($10, 15 \mu\text{g/mL}$) treatment. These observations indicated that activation of caspase cascade might play a crucial role in AGB-5 induced apoptotic death in MCF-7 cells.

Effect of AGB-5 on Tumor Growth As shown in Table 1, at the doses of 3, 6, and 12 mg/kg , AGB-5 significantly reduced the tumor volume as compared to that of MCF-7 control group ($p < 0.01$, $p < 0.001$). At the dose of 6 mg/kg , AGB-5 showed maximum efficacy, which was similar with the positive control (DDP, 2 mg/kg). While the 12 mg/kg -treated group showed lower activity than 6 mg/kg -treated group. The body weights of mice in this group were also visibly increased. These data proved that AGB-5 also exerted a preferable proliferation inhibition effect *in vivo*.

Effect of AGB-5 on Hematological Parameters As shown in Table 2, hemoglobin content and RBC count de-

Fig. 3. Investigation of Cell Cycle Distribution of MCF-7 Cells Treated by AGB-5

The cells treated with 0, 5 and $10 \mu\text{g/mL}$ AGB-5 for 24 h were collected and stained with PI and analyzed by flow cytometry. $**p < 0.01$ compared with control.

Fig. 4. AGB-5 Induced Apoptosis in MCF-7 Cells

(A) MCF-7 cells were incubated with AGB-5 (0, 5, 10 µg/mL) for 24h. Apoptosis was analyzed by staining phosphatidylserine translocation with Annexin V-FITC. (B) The morphological changes were examined by Hoechst 33258 staining under fluorescence microscopy (×200). The results shown here are representative of three independent experiments.

Table 1. Effect of the Ethanol Extract of *Ardisia gigantifolia* STAFF. Rhizome (AGB-5) on Body Weight and Tumor Volume of MCF-7 Bearing Mice

Parameters	MCF-7 control (2×10 ⁶ cells/mouse)	AGB-5 3 mg/kg +MCF-7	AGB-5 6 mg/kg +MCF-7	AGB-5 12 mg/kg +MCF-7	DDP 2 mg/kg +MCF-7
Body weight (g)	18.55±0.9	18.64±0.78	18.45±0.63	19.4±0.93	19.45±1.54
Tumor volume (mL)	0.9±0.10	0.7±0.05**	0.55±0.08***	0.6±0.06***	0.5±0.03***

Data are expressed as the mean of results in 6 mice±S.E.M. Extract-treated groups was compared with the MCF-7 control group. ** $p<0.01$, *** $p<0.001$.

Table 2. Effect of the Ethanol Extract of *Ardisia gigantifolia* STAFF. Rhizome (AGB-5) on Hematological Parameters of MCF-7 Bearing Mice

Parameters	Normal	MCF-7 control (2×10 ⁶ cells/mouse)	AGB-5 3 mg/kg +MCF-7	AGB-5 6 mg/kg +MCF-7	AGB-5 12 mg/kg +MCF-7	DDP (cisplatin) 2 mg/kg +MCF-7
Hemoglobin (g/L)	158±0.97	104±0.82 [#]	99±0.79***	109±1.08***	116±1.04***	134±2.80***
RBC (×10 ¹² /L)	10.05±0.21	8.01±0.58 [#]	8.90±0.11*	9.84±0.37***	9.10±0.41**	9.75±0.39***
WBC (×10 ⁹ /L)	5.30±0.42	11.40±0.38 [#]	7.8±0.25***	7.45±0.31	6.60±0.18***	6.98±0.24***

Data are expressed as the mean of results in 6 mice±S.E.M. The MCF-7 control group was compared with normal group where [#] $p<0.001$. Extract-treated groups was compared with the MCF-7 control group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

creased significantly and the total WBC count increased significantly in MCF-7 group as compared to normal group ($p<0.05$). Interestingly, treatment with AGB-5 restored the hematological parameters to near normal values. The RBC count and hemoglobin content significantly increased, while the WBC count significantly decreased in the AGB-5 and standard drug treated groups when compared to that of MCF-7 control ($p<0.001$).

Effect on Antioxidant Enzymes (SOD and Catalase)

The inoculation of MCF-7 cells caused significant decreases in the levels of the antioxidant enzymes SOD and CAT when compared to the normal group (Table 3). As shown in Table 3, administration of AGB-5 at doses of 6 and 12 mg/kg signifi-

cantly increased the level of SOD, respectively, as compared to that of the MCF-7 control group ($p<0.05$). Treatment with AGB-5 at doses of 3, 6, and 12 mg/kg significantly increased catalase levels ($p<0.01$, $p<0.001$), respectively, when compared to that of the MCF-7 control. At the dose of 2 mg/kg, cisplatin (DDP, sigma, >99%) similarly increased the SOD and catalase level (Table 3).

DISCUSSION

Perturbation of cell cycle progression in cancer cells is a useful strategy to arrest cancer growth.¹⁷⁾ Furthermore, cell cycle arrest also provides an occasion for cells to undergo ei-

Table 3. Effect of Ethanol Extract of *Ardisia gigantifolia* STAFF. Rhizome (AGB-5) on Liver Enzymes of MCF-7 Bearing Mice

Parameters	Normal	MCF-7 control (2×10 ⁶ cells/mouse)	AGB-5 3 mg/kg +MCF-7	AGB-5 6 mg/kg +MCF-7	AGB-5 12 mg/kg +MCF-7	DDP (cisplatin) 2 mg/kg +MCF-7
SOD (U/mg protein)	31.25±0.95	28.73±1.09 ^{##}	29.59±1.40	30.66±1.28*	30.1±1.00*	30.12±0.95*
CAT (U/mg protein)	0.23±0.019	0.126±0.015 ^{###}	0.174±0.013 ^{***}	0.181±0.034 ^{**}	0.20±0.095 ^{***}	0.147±0.016*

Data are expressed as the mean of results in 6 mice±S.E.M. MCF-7 control group was compared with normal group. ^{##}*p*<0.01, ^{###}*p*<0.001. Extract-treated groups were compared with the MCF-7 control group. **p*<0.05, ***p*<0.01, ****p*<0.001.

ther repair or programmed cell death. At the dosage of 10 µg/mL, AGB-5 can significantly increase S phase, decrease G2/M phase and have no influence on G1 phase compared with the control group. The varying effects of AGB-5 on the cell cycle may be due to cell-type specificity and/or result from modulation of different signal transductions and cell cycle regulatory molecules. Apoptosis seems to be a reliable marker for the appraisal of potential agents for cancer prevention, and a wide variety of natural products have been known to have the ability to induce apoptosis in various human tumor cells.¹⁸⁾ Therefore, to investigate the anti-proliferative effects of AGB-5 on MCF-7 cells, apoptosis analysis was determined. As shown in Fig. 4A apoptotic MCF-7 cells significantly increased in a dose-dependent manner after treatment with AGB-5. In addition, the conspicuous changes in MCF-7 cell nuclear morphology could be the direct reflections of cell apoptosis (Fig. 4B). A molecular hallmark of apoptosis is the activation of caspases, which has been demonstrated to play a central role during cellular apoptosis.^{19,20)} Caspase-3 is a prevalent caspase that is ultimately responsible for the majority of apoptotic processes and can be activated by upstream initiator caspases such as caspase-9 through the mitochondria dependent-cytochrome *c*/caspase-9 intrinsic pathway.²¹⁾ Therefore, we examined the effects of AGB-5 on the activation of caspase-3, and -9 by spectrophotometry. As shown in Fig. 5, the activity of caspase-3 and -9 were significantly increased in a dose-dependent manner in AGB-5 treated MCF-7 cells. These results suggest that caspase-9 and -3 play pivotal roles in AGB-5 induced apoptosis in MCF-7 cells.

The reliable criteria for judging the value of any anticancer drug is the prolongation of life span of the animals and the decrease of leukemic cells from blood.¹⁴⁾ When compared to the MCF-7 control group, AGB-5 significantly decreased tumor volume without affecting the bodyweight. Hence, AGB-5 may increased the life span of MCF-7-bearing mice through direct cytotoxic effect and tumor growth inhibition. In cancer chemotherapy the major problems are myelosuppression and anemia.²²⁾ The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or hemoglobin percentage and this may occur either due to iron deficiency or due to hemolytic or myelopathic conditions.²³⁾ Similar results were observed in the present study in animals of the MCF-7 tumor control group. The reversal of RBC and WBC count indicates that AGB-5 may possess protective action on the hematopoietic system. This reinstates that use of herbs might be a more effective strategy in the treatment of cancer.

It was observed that tumor cells produced more peroxides when they proliferate actively after inoculation of tumor and also known to affect many functions of the vital organs, which indicated the intensification of oxygen free radical production. The elevation of lipid peroxidation is also known to

Fig. 5. Effect of AGB-5 on Caspase-3 and -9 Activities in MCF-7 Cell Lines

After 24h treatment with or without AGB-5, caspase-3 and -9 activities were determined by mixing the cell lysates with colorimetric substrate (DEVD-pNA, LEHD-pNA). The absorbance was measured at 405 nm. Data are mean±S.E.M. (*n*=6). **p*<0.05 and ***p*<0.01 compared with control.

be associated with cancer.²⁴⁾ Decrease of SOD, GSH and CAT levels is regarded as markers of malignant transformation.²⁵⁾ Therefore the significant elevation of SOD and CAT by the extract treatment confirms the potent antioxidant activity and free radical quenching property of AGB-5.

In conclusion, we observed that AGB-5 significantly reduces cancer cell growth in both *in vitro* and *in vivo* models. However, the exact mechanism behind the antitumor activity of AGB-5 is beyond the scope of present study. Though, protecting the hemopoietic system and elevating the endogenous antioxidant enzyme may have played an important role.

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