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Aloe vera gel extract: Safety evaluation for acute and chronic oral administration in Sprague-Dawley rats and anticancer activity in breast and lung cancer cells

Xueli Tong [a], Min Li [a], Di Li [a], Chunqin Lao [a], Jingmian Chen [a], Weijie Xu [a], Junxi Du [a], Meijiao Zhang [b], Xiangcai Yang [a,*], Jiejing Li [a,**]

[a] Department of R&D, Biotech&Science Company of UP, Ltd., Guangzhou, 510000, China
[b] Department of Drug Safety Evaluation Center, Guangzhou University of Chinese Medicine Science and Technology Industrial Park Co., Ltd., Guangzhou, 510000, China

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ABSTRACT

Ethnopharmacological relevance: Aloe vera (L.) Burm. f. is a typical traditional Chinese medicine (TCM) collected in the Pharmacopoeia of the People's Republic of China (version 2015). It has been traditionally used for the treatment of constipation, and its potential therapeutic activities have been widely evaluated, including anti-tumor, anti-inflammatory and immune regulatory effects. The wide application of *Aloe vera* in food and therapy has raised safety issues and there are multiple safety assessments with a diverse toxicity and adverse effects from clinics and animals.

Aim of the study: This study aimed to investigate the safety of *Aloe vera* barbadensis extract C (AVBEC) in rats and analyze its anticancer activity in cell lines.

Materials and methods: We administrated AVBEC orally in an acute toxicity study and a 6-month chronic toxicity study to observe and confirm its safety in Sprague-Dawley (SD) rats. Additionally, we explored the cytotoxicity of AVBEC in cancer cells and non-cancer cells. We further investigated the anti-tumor activity of AVBEC, and in the meantime, probed the function of component from AVBEC.

Results: No deaths or substance-relative toxicity were observed in the acute toxicity study or the 6-month chronic toxicity study with doses of 44.8 $g \cdot kg^{-1}$ and 4.48 $g \cdot kg^{-1}$, respectively. In the chronic toxicity study, AVBEC did not cause organ toxicity, including crucial organ structure and chemical function, and peripheral and central immune system damage. Additionally, we found that AVBEC could induce cancer cell apoptosis with a relatively higher apoptotic ratio than in non-cancer cells by decreasing adenosine triphosphate (ATP) concentration and enhancing reactive oxygen species (ROS) production. We also identified components in AVBEC using high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) and probed the function of malic acid. This demonstrated that under the same circumstances, malic acid induced cell necrosis in cancer cells and non-cancer cells, while AVBEC did not.

Conclusions: These results reveal a novel mechanism of aloe gel extract in regulating cancer cell apoptosis *via* modulating the mitochondrial metabolism and imply a possible application of AVBEC for the treatment of

Abbreviations: AVBEC, *Aloe vera barbadensis* extract C; AMPK, adenosine 5′-monophosphate-activated protein kinase; ATP, adenosine triphosphate; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; ALB, albumin; APTT, activated partial thromboplastin time; BASO, basophil; BIL, bilirubin; CFDA, China Food and Drug Administration; Cl^-, chloride; CK, creatine kinase; CRE, creatinine; CHOL, total cholesterol; DCFH-DA, 2,7-Dichloro-dihydro-fluorescein diacetate; EOS, eosinophil; ER, endoplasmic reticulum; FITC, fluorescein isothiocyanate; GGT, γ-glutamyltransferase; GLU, glucose; HPLC-MS/MS, high performance liquid chromatography-tandem mass spectrometry; HCD, higher-energy C-trap dissociation; HGB, hemoglobin; HCT, hematocrit level; K^+, potassium; KET, ketone bodies; LYM, lymphocyte; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MONO, monocyte; NIT, nitrite; NEUT, neutrophil; Na^+, sodium; OB, occult blood; PI, propidium iodide; PerCP, Peridinin-Chlorophyll-Protein Complex; PLT, platelet; PT, prothrombin time; PRO, protein; ROS, reactive oxygen species; RETIC, reticulocyte; RBC, red blood cell; SD, Sprague-Dawley; SG, specific gravity; TCA, tricarboxylic acid cycle; TCM, Traditional Chinese Medicine; TBIL, total bilirubin; TP, total protein; TG, triglycerides; Urea, urea nitrogen; URO, urobilinogen; WBC, white blood cell; 7-AAD, 7-amino-actinomycin D.

* Corresponding author. Department of R&D, Biotech&Science Company of UP, Ltd., 918 Guangzhou Avenue, Guangzhou, Guangdong, 510000, China.
** Corresponding author. Department of R&D, Biotech&Science Company of UP, Ltd., 918 Guangzhou Avenue, Guangzhou, Guangdong, 510000, China.
E-mail addresses: shuiyuejueshi@126.com (X. Yang), xenjiejingli@hotmail.com (J. Li).

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malignant cancer with the safety evaluation from rats and anticancer investigation from cancer cells and non-cancer cells.

1. Introduction

Aloe vera (L.) Burm. f. (synonym *Aloe barbadensis* Mill.) is a traditional Chinese medicine (TCM) herb catalogued in the Pharmacopoeia of the People's Republic of China (version 2015). *Aloe vera* has been widely used as a therapeutic agent and food for over 2000 years in Egypt, Greece and China, and its traditional applications include constipation, wound healing and anti-tumor (Hekmatpou et al., 2019; Radha and Laxmipriya, 2015; Zagórska-Dziok et al., 2017), which is also indicated in Chinese Materia Medica (version 1999). Some bioactive compounds enriched in *Aloe vera* are anthraquinones from aloe latex, including aloin and emodin, and polysaccharides from aloe gel, including glucomannan and ace-mannan. Accumulating preclinical evidence demonstrates that those compounds from *Aloe vera* have anticancer, anti-inflammatory, wound healing and immune modulation functions (Budai et al., 2013; Lopez et al., 2019; Majumder et al., 2019).

Cancer is a major public health issue worldwide, and lung cancer and breast cancer are the most frequently diagnosed and leading causes of cancer-related mortality in men and women respectively (Sung et al., 2021). Considering the rapidly growing burden of cancer mortality, the development of novel anticancer drugs with selective targeting for cancer cells and low toxicity for normal cells remains a key research topic. Accumulated studies have demonstrated that *Aloe vera* can function as a potential therapeutic agent for various cancers, including breast and lung cancers (Majumder et al., 2019). The anticancer effects of *Aloe vera* may be mediated coordinately by its bioactive compounds, including polysaccharides and anthraquinones. Polysaccharides, especially ace-mannan, have immunomodulatory activity (Liu et al., 2019), including anti-inflammation (Budai et al., 2013), immune activation (Harlev et al., 2012; Kumar and Tiku, 2015), including pluripotent effector cells, macrophage. Anthraquinones, including emodin and aloin, have been well researched for their antitumor effects. This has drawn attention to their chemopreventive and radioprotective potential (Haddad et al., 2013; Hamiza et al., 2013), anti-proliferative and apoptosis-inducing potential with or without anticancer agents (Hussain et al., 2015; Im et al., 2016; Lee et al., 2014a,b; Sanders et al., 2017). From the clinical trials, *Aloe vera*, as a complementary therapeutic product, was proven to be effective for the alleviation of side effects induced by chemotherapy and radiotherapy, and therefore substantially facilitates patients to recover (Farrugia et al., 2019; Mansouri et al., 2016). For instance, some clinical trials have shown that *Aloe vera* is beneficial to alleviate acute proctitis and dermatitis occurs after radiation (Haddad et al., 2013; Sahebnasagh et al., 2017). Besides, Damani et al. reported the application of *Aloe vera* eye drops in a 64-year-old patient induced the regression and finally resolved the ocular surface squamous neoplasia (Damani et al., 2015).

However, due to the underestimation of toxicity and insufficient safety evaluation, the consumption of *Aloe vera* often raises some safety issues. Currently, with the widespread medical application of *Aloe vera*, an exhaustive investigation for the toxicity and other relevant adverse impacts are required, and there were plenty of clinical trials already focusing on the study of this area. Hepatic toxicity is one of the most common adverse effects of herbal supplement use (Lin et al., 2019). In some previous cases, patients identically exhibited toxic hepatitis or liver injury with abnormalities in liver enzymes and histopathology with oral *Aloe vera* products, nevertheless, the patients recovered after discontinuation of the *Aloe vera* administration (Lee et al., 2014a,b; Parlati et al., 2017; Yang et al., 2010). As a type of laxative, the application of *Aloe vera* latex also resulted in functional damage, such as colorectal lesions and kidney dysfunction (Cholongitas et al., 2005; GORKOM et al., 1999). Although there were no severe adverse effects or carcinogenicity reported owing to the application of *Aloe vera* gel, the occurrence of allergy or hypersensitivity of skin might be an obstacle limiting the extensive use for this specific portion of it (Ferreira et al., 2007; Morrow et al., 1980). Overall, the current state of *Aloe vera* application is a controversial issue. On the one hand, the benefits of *Aloe vera* indicated that it could have a high potential to be used as an excellent therapeutic agent for numerous diseases. On the other hand, the safety issues have impeded the further application of *Aloe vera*. Under these circumstances, a thorough safety evaluation is needed to offer a deeper insight into the judgement of toxicity of *Aloe vera* and facilitate the application of *Aloe vera* products.

Commonly, *Aloe vera* gel has been used as concentrated powder with the application of organic solvent and lyophilization/vacuum evaporation during the procedure. The gel powder should be dissolved to a certain concentration when it was subject to analysis for functional investigation or clinical treatment (Huseini et al., 2012; López et al., 2017; Rajasekaran et al., 2005; Rathor et al., 2012). Here, we obtained an *Aloe vera* gel extract named AVBEC (*Aloe vera barbadensis* extract C) solution without supplement of organic solvent and lyophilization/vacuum evaporation for dehydration to powder during the procedure. Most importantly, we presented the chemical constituents of malic acid, citric/isocitric acid by HPLC-MS/MS and several polysaccharides (isolated with column chromatography and identified with ^{1}H-NMR and ^{13}C-NMR, data not shown here). These components are the quality control markers of *Aloe vera* products (Jiao et al., 2010), and also the essential bioactives, which synergistically act in the therapeutic activities of *Aloe vera*. In the current study, we evaluated the safety of AVBEC *via* acute and chronic (6-month) toxicity studies in Sprague-Dawley (SD) rats. In addition, we have found that AVBEC could induce a relative higher ratio of cell apoptosis in breast cancer (MCF7 and MDA-MB-231 with different malignancies) and lung cancer cells (small cell lung cancer cell NCI-H 524 and non-small cell lung cancer cell NCI-H 1975) than non-cancer cells (HEK293), which might be mediated by modulating mitochondrial metabolism. This is the first safety evaluation in both human cell lines and animals (6-month chronic test) of *Aloe vera* gel extract, and investigation of its effect on cancer cells.

2. Material and methods

2.1. Plant materials and sample preparation

Leaves of *Aloe vera* (L.) Burm. f. were purchased (batch number: 20180806) from Yunnan Evergreen Biological Corporation, Yuanjiang (Latitude: 23°62′N, Longitude: 101°98′E), Yunnan Province, China. The obtained leaves were identified by Dr. Tian from school of Southern Medical University, in compliance with the quality standard of the Chinese Pharmacopoeia (version 2015). A voucher specimen was deposited in the Department of R&D, Biotech&Science Company of UP (No. UP-R&D-20180811).

The freshly collected aloe leaves were washed with distilled water, water was removed under ventilated conditions and the leaves were peeled. The AVBEC was obtained with fresh gel by homogenization, and the debris was removed by centrifugation at 300 *g* for 3 min, mixed with 3 vol of water and stirred at low speed at 60 °C for 3 times (2 h per time), sterilized with autoclave, concentrated to a suitable volume under reduced pressure and filtered again. The yield of AVBEC from *Aloe vera* gel was 12.5%, with a density of 1.12 g mL^{-1} and was stored at 4 °C. The compositions of AVBEC were detected by HPLC-MS/MS after freeze-drying. For the animal experiments, the storage solution was ready for use. For the cell culture, the stock solution was diluted to the appropriate concentration by the corresponding culture medium and then filtered

with a 0.22 μm sterilizing membrane filter before use.

2.2. HPLC-MS/MS analysis

The contents of AVBEC were detected using HPLC-MS/MS (Thermo, Ultimate 3000LC, Q Exactive HF) equipped with a Zorbax Eclipse C18 (1.8 μm*2.1 mm*100 mm, Agilent) after multiple pre-processing steps. In brief, AVBEC was analyzed after the processes of freeze-drying, grinding, dissolution, centrifugation, and filtration. The mobile phase was as follows: solvent A, acetonitrile, and solvent B, H_2O containing 0.1% formic acid. The gradient elution was 0–2 min, 5% (A), 2–6 min, 5–30% (A), 6–7 min, 30% (A), 7–12 min, 30–78% (A), 12–14 min, 78% (A), 14–17 min, 78–95% (A), 17–18 min, 95% (A). Other important parameters are the flow rate of 300 μL/min, injection volume of 2 μL, source temperature of 325 °C, the ESI voltage of 3.5 kV, sheath gas flow rate of 45 arb, and auxiliary gas flow rate of 15 arb. The data were obtained and analyzed using available MS/MS databases including Thermo mzCloud and Thermo mzValut.

2.3. Safety evaluation of AVBEC in SD rats

2.3.1. Experiment animals

The experimental procedures followed the good laboratory practices (GLP) and were authorized by the Institutional Animal Care and Use Committee of the Sci-tech Industrial Park, Guangzhou University of Chinese Medicine. The approval numbers for acute toxicity study and chronic toxicity studies were PZ19071 of and PZ19072, respectively.

SD rats were purchased from the Guangdong Medical Laboratory Animal Center (SCXK [Guangdong] 2018-0002). In the acute toxicity study, the weights of male and female SD rats (7–8 weeks old) ranged from 180 to 200 g and 140–180 g, respectively. In the chronic toxicity study, the weights of male and female SD rats (6–7 weeks old) ranged from 140 to 170 g and 130–160 g, respectively. All animals were housed at the animal facility with a 12 h dark and 12 h light cycle, 40–70% humidity, 22 ± 2 °C temperature (SYXK [Guangdong] 2018-0014). All animals were quarantined and raised for five days to adapt to the altered conditions before the experiments.

For some low-toxicity substances, the maximum feasible dose (MFD) can be used to carry out the acute toxicity tests. It is currently believed that the maximum volume of intragastric gavage for rats is approximately 40 ml kg^{-1} (Diehl et al., 2001). In the acute toxicity study, rats were administered 20 ml kg^{-1} AVBEC twice within 24 h to evaluate its acute toxicity. Therefore, the dose of AVBEC given to each rat was 40 ml kg^{-1}, which is equivalent to 44.8 g kg^{-1} (the density of AVBEC: 1.12 g mL^{-1}).

According to the technical guidelines for repeated drug dose toxicity testing (20140513, SFDA), the high, medium and low doses are commonly set to 1/10 LD50, 1/50 LD50 and 1/100 LD50, respectively (Li et al., 2020). Acute toxicity testing showed that there was no mortality or toxic reaction due to the addition of AVBEC during the experiment. In our chronic toxicity experiment, the dosage of the high-dose group was set 4.48 g kg^{-1} (1/10 of the MFD in the acute toxicity study).

2.3.2. Acute toxicity study

The acute oral toxicity study of AVBEC was evaluated following the CFDA guidelines (20140513). We selected 40 SD rats from the available rats for the acute toxicity test. The SD rats were randomly divided into two groups of 20 (equally divided by sex): negative control group (treated with purified water) and AVBEC treated group (44.8 g kg^{-1}). All rats were fasted overnight prior to the intragastric administration. Two groups were separately administered of water or AVBEC twice *via* intragastric gavage (20 ml kg^{-1}) in the span of approximately 4 h. Animal were allowed food approximately 2 h after the first administration. The rats were closely monitored for 4 h after the dose administration. The day when the rats were exposed to the test substance was recorded as D0. General health observations (daily), mortality (daily), weight (once a week), and symptoms of poisoning (daily) were monitored and registered for two weeks. The observation period of two weeks' post-treatment was recorded as rD1 to rD14 (recovery period). The rats were sacrificed and examined for histopathological changes at rD14.

2.3.3. 6-Month chronic toxicity study

The 6-month chronic toxicity of AVBEC was evaluated according to the CFDA guidelines (20140513). We selected 200 SD rats from the available rats for the chronic toxicity test. The SD rats were randomly divided into four groups of 50 (25 of each sex): negative control group (treated with purified water), low-dose group (treated with 1.12 g $kg^{-1}\cdot d^{-1}$ AVBEC), medium-dose group (treated with 2.24 g $kg^{-1}\cdot d^{-1}$ AVBEC) and high-dose group (treated with 4.48 g $kg^{-1}\cdot d^{-1}$ AVBEC). The rats were given these experimental treatments as described above at every morning for six months and the corresponding period was recorded as D1-D181. In addition, there was a four-week observation period after the final administration which was recorded as rD1 to rD29. General health observations were conducted daily, and the weights and food intakes for each survival rat were recorded once per week. The volume of AVBEC was adjusted according to the weights of the rats. At the mid-term administration period (D90), 50 rats (half of each sex) among each group were fasted for 12 h, and their urine samples were collected. On the next day (D91), 20 rats were sacrificed and three blood samples were collected from the abdominal aorta to conduct a hematologic analysis (EDTA-K2 tubes), a biochemistry analysis (dry tubes) and coagulation tests (sodium citrate tubes). Vital organs, including the heart, liver and kidney, were excised, weighed and fixed in 10% formaldehyde while the testis were uniquely fixed in Davidson fixative solution for histopathological examination after euthanasia. The femur marrow was used for the bone marrow smear. Similarly, the urine of 30 rats and the remaining 10 rats for each group were collected at the end of the administration period (D181) and the last day of the recovery period (rD28), respectively. The rats were sacrificed on the first day of the recovery period (rD1, 20 rats) and the end of the recovery period (rD29, 10 rats), respectively.

2.3.3.1. Blood biochemistry analysis. For the blood biochemistry analysis, the blood samples collected with dry tubes were rested for 30 min in a constant-temperature tank at 37 °C. Then the samples were centrifuged at 3000 rpm for 10 min and the supernatant was carefully transferred to another cleaning tube. Alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), creatine kinase (CK), creatinine (CRE), total bilirubin (TBIL), γ-glutamyltransferase (GGT), albumin (ALB), total protein (TP), urea nitrogen (urea), total cholesterol (CHOL), glucose (GLU), and triglycerides (TG) were detected using a Hitachi 7080 analyzer, while potassium (K^+), sodium (Na^+), chloride (Cl^-) were detected using a MEDICA Easylyte analyzer (MEDICA, USA).

2.3.3.2. Urinalysis

Urine samples were collected using stress stimulation. Glucose (GLU), protein (PRO), pH, occult blood (OB), ketone bodies (KET), bilirubin (BIL), urobilinogen (URO), specific gravity (SG), white blood cells (WBC), and nitrite (NIT) were detected by CLINITEK100 (BAYER, Germany).

2.3.3.3. Hematologic analysis

For the hematologic analysis, the samples were collected in EDTA-K2 tubes and analyzed within 30 min. The white blood cell count (WBC), neutrophil ratio (%NEUT), lymphocyte ratio (%LYM), monocyte ratio (%MONO), eosinophil ratio (%EOS), basophil ratio (%BASO), red blood cell count (RBC), hemoglobin (HGB), hematocrit level (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),

mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and reticulocyte ratio (%RETIC) were examined using an ADVIA2120 automatic cell analyzer (Siemens, Germany). Samples collected with sodium citrate-containing tubes were used for the coagulation test. The prothrombin time (PT) and activated partial thromboplastin time (APTT) were measured using a Sysmex CA1500 (SYSMEX, Japan).

2.3.3.4. Histological analysis and bone-marrow smear

The fixed organs were dehydrated in gradient ethanol, vitrified, and embedded in paraffin. Sections (5 μm thick) were cut with an RM2235 microtome (Leica, Germany) and stained with hematoxylin and eosin. Bone marrow from the femur was smeared. Histological observations were performed using a Leica DMLB bright field microscope (Leica, Germany) at 200 and 400 magnification to evaluate the degree of pathological alteration. Pathological evaluation of the tissue sections was performed by an experienced pathologist.

2.4. Investigation of AVBEC in human cell lines

2.4.1. Cell culture

Four cancer cell lines (MCF-7, MDA-MB-231, NCI-H 524, NCI-H 1975) and normal human embryonic kidney cells (HEK293) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). MDA-MB-231 and HEK293 cells were cultured in Dulbecco's modified Eagle's medium and the other cell lines were maintained in RPMI-1640 medium, containing 10% fetal bovine serum and 1% penicillin/streptomycin in a 5% CO_2 atmosphere at 37 °C temperature. With the exception of the conditions mentioned above, 1% insulin transferrin-selenium was required for the cultivation of NCI-H 524.

2.4.2. Cell viability analysis (CCK-8 assay)

Cells were seeded in 96-well plates at an appropriate density overnight. The medium was replaced with fresh medium containing or not AVBEC at a concentration of 2.5, 5, and 10 mg/ml and the cells were further cultured for 24 h. After treatment, CCK-8 solution (C0038, Beyotime) was added to each well and the 96-well plate was incubated at 37 °C for another 4 h. The OD_{450} values were recorded using a microplate reader (SynergyTM LX, BioTek, USA). The cell viability was then calculated.

2.4.3. Cell apoptosis analysis

Cells were plated in 12-well plates and ensured that the density of the next day can reach about 70%. After incubation overnight, the medium was replaced by fresh medium with AVBEC at a concentration of 2.5, 5, and 10 mg/ml for 8 h. Then, we detected the apoptosis rate following the instructions of the protocol of FITC-Annexin V apoptosis Detection Kit (556547, BD Biosciences). After that, the cells were treated with 10 mg/ml AVBEC for different durations, and the apoptosis was detected to assess the relationship between dose and effect.

2.4.4. Western blotting

Cells were seeded in 6-well plates and treated with AVBEC (10 mg/ml) for different durations, which was determined by the rate of apoptosis in different cell lines. Total protein was collected from the cell lysates. Western blotting was used to quantify the expression of cleaved caspase-3 (1:1000, 9661S, Cell Signaling Technology), β-actin (1:10,000; ab6276, Abcam). Detection was performed using horseradish peroxidase (HRP)-conjugated secondary antibodies. The blots were visualized by ECL and measured using ImageJ software.

2.4.5. Apoptosis and necrosis analysis

Malic acid (HPLC≥98%, B20937) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. The concentration of malic acid in AVBEC was 36.06%, as detected by HPLC-MS/MS. Malic acid was always freshly dissolved in the culture medium before use.

Cells were cultured in 6-well plates and treated with 10 mg/ml AVBEC or 3.6 mg/ml malic acid (10 mg AVBEC containing malic acid 3.6 mg) for 30 min. Cells were collected and stained with Apopxin green and 7-AAD dye for 30 min to indicate the cells in early apoptosis, late apoptosis and necrosis according to the description of apoptosis/necrosis detection kit (ab176749, Abcam). Apoptosis was measured with Apopxin Green by using FITC channel and the necrosis of cells was measured with 7-AAD by using the PerCP channel.

2.4.6. Intracellular ROS determination

Cells were plated in 12-well plates as mentioned above and treated with 10 mg/ml AVBEC for 8 h. The determination process was conducted according to the instructions of the Reactive Oxygen Species Assay Kit (CA1410, Solarbio). The cells were labeled with DCFH-DA for 30 min. The cells were then washed thrice with PBS. The cells were collected to detect the fluorescence intensity of DCF using flow cytometry at Ex/Em = 488/525 nm. Meanwhile, cells plated in chamber slides were imaged immediately after labeling with DCFH-DA by fluorescence microscopy.

2.4.7. ATP analysis

Cells were cultured in T75 culture flasks and treated with 10 mg/ml AVBEC for 8 h. Cells (1×10^6 cells) were collected, snaped-frozen in liquid nitrogen for 1 s, and immediately stored at −80 °C. After collecting samples from the four cell lines, the ATP concentrations were quantified according to the instructions of the ATP assay kit (ad83355, Abcam).

2.5. Statistical analysis

The data were processed using SPSS19.0 and expressed as mean ± SD. If the data met the normal distribution, the statistical significance between groups was determined by one-way ANOVA, *t*-test or SNK-q. If it did not meet the normal distribution and the homogeneity of variance test, the Kruskal-Wallis H test was used.

3. Results

3.1. HPLC-MS/MS analysis of AVBEC

We identified the components of AVBEC by applying high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) in higher-energy C-trap dissociation (HCD) positive mode and negative mode (Fig. 1A, B). After analyzing the data, we obtained 50 compounds in the AVBEC. As shown in Fig. 1C and Table S1, the top five components were malic acid (36.06%), gluconic acid (16.63%), lactobionic acid (6.34%), isocitric acid (5.35%), citric acid (5.08%).

3.2. Safety evaluation of AVBEC in SD rats

3.2.1. Acute toxicity study of AVBEC

After 14-days observation period, no abnormal clinical signs or deaths occurred in SD rats with the AVBEC treatment. Body weight was measured at D0, rD1, rD7, and rD14, and there was no statistically significant difference between the AVBEC-treated and non-treated groups ($P > 0.05$, Fig. 2A, C, and D). At the end of the acute administration evaluation, pathological examination showed no obvious lesions or abnormalities in the volume, color, or texture of isolated organs due to the treatment with AVBEC (data not shown).

3.2.2. 6-Month chronic toxicity study of AVBEC

We performed ophthalmology and urine examinations on rats at the mid-term administration period (D90), the end of the administration

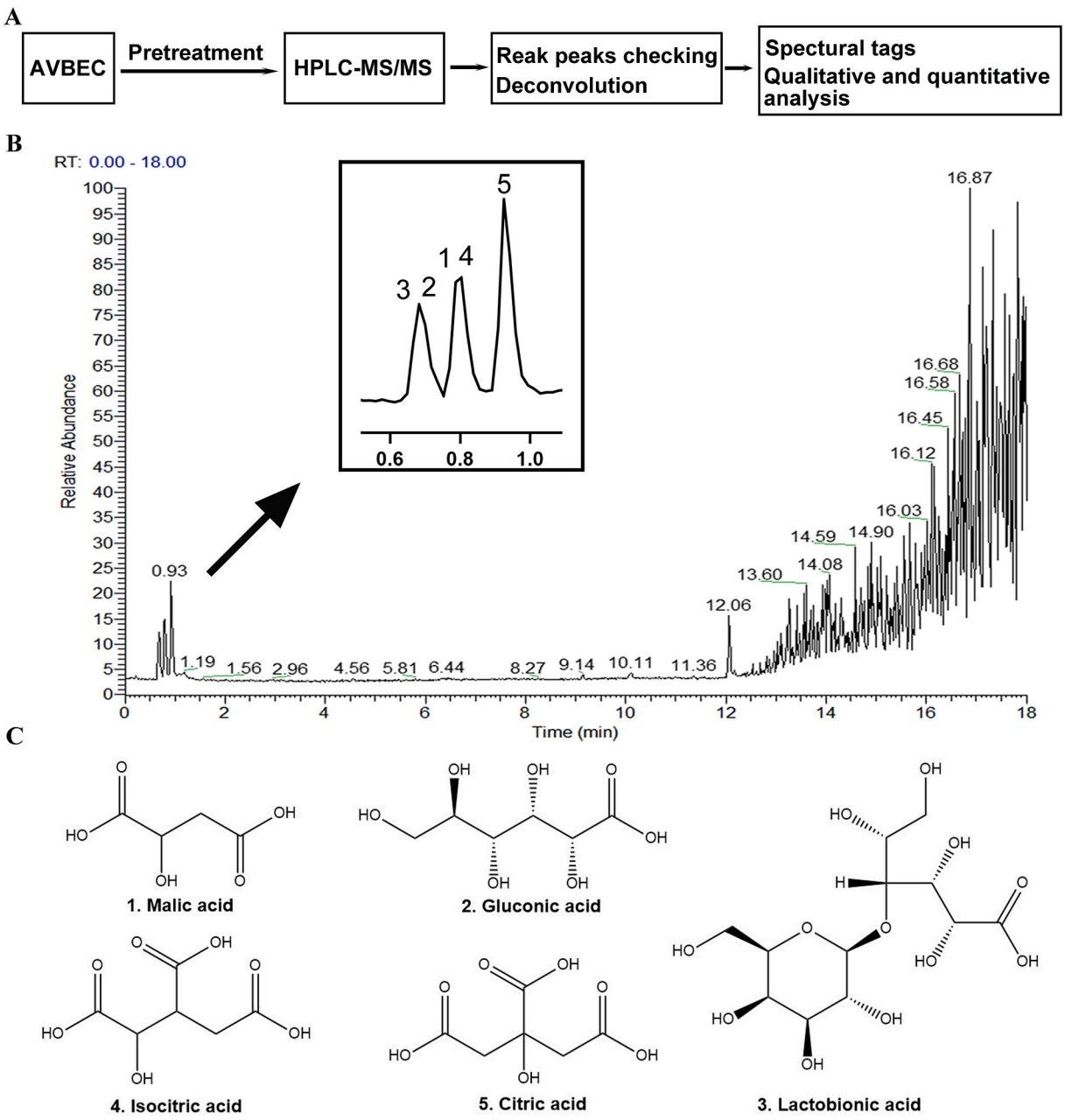

Fig. 1. HPLC-MS/MS analysis of AVBEC. (A) Main procedures of analyzing AVBEC composition using HPLC-MS/MS. (B) Total ion chromatography of negative mode. Numbers 1–5 represent the peak position of the five compounds with the highest content in AVBEC. (C) Structures of the five compounds with the highest content in AVBEC.

period (D181) and the recovery period (rD28), and the hematology, blood biochemical assay and histopathological examinations were performed at D91, rD1 and rD29 (Fig. 2B). From the chronic toxicity testing, there was no mortality or toxic pathological damage to tissues and organs occurred due to the addition of AVBEC. We considered the lack of toxicological significance with some changes in blood biochemistry analysis, urinalysis and peripheral blood assay from AVBEC-treated groups because they are neither time-dependent nor dose-dependent (see Table S2-5). Additionally, there was no abnormality in rats after the recovery period. We provide representative data from the negative control and high-dose groups.

3.2.2.1. Body weight and food consumption. There was no significant difference between the high-dose AVBEC-treated group and the negative control group in body weight (Fig. 2E and F). The food consumption for some rats in the high-dose group was comparatively low, which could be considered as a transient change because there was no significant difference in body weight between the AVBEC-treated group and the control group during the same period (Fig. 2G and H).

3.2.2.2. Organ chemistry. First, the organ coefficients, which is a common toxicology indicator reflecting animal damage from drug testing, showed that there was no difference between the two groups, indicating that no obvious organ damage was caused by the addition of AVBEC (Table S2), and hematoxylin-eosin (H&E) staining of crucial organs, including the liver (Fig. 3A–F), kidney (Fig. 3G-L) and heart (Fig. 3M–R), manifested that there was no pathological injury displayed in the histologic structure at different stages during the repeated treatment of AVBEC, which emphasized the non-toxicity of AVBEC for organs in a certain duration.

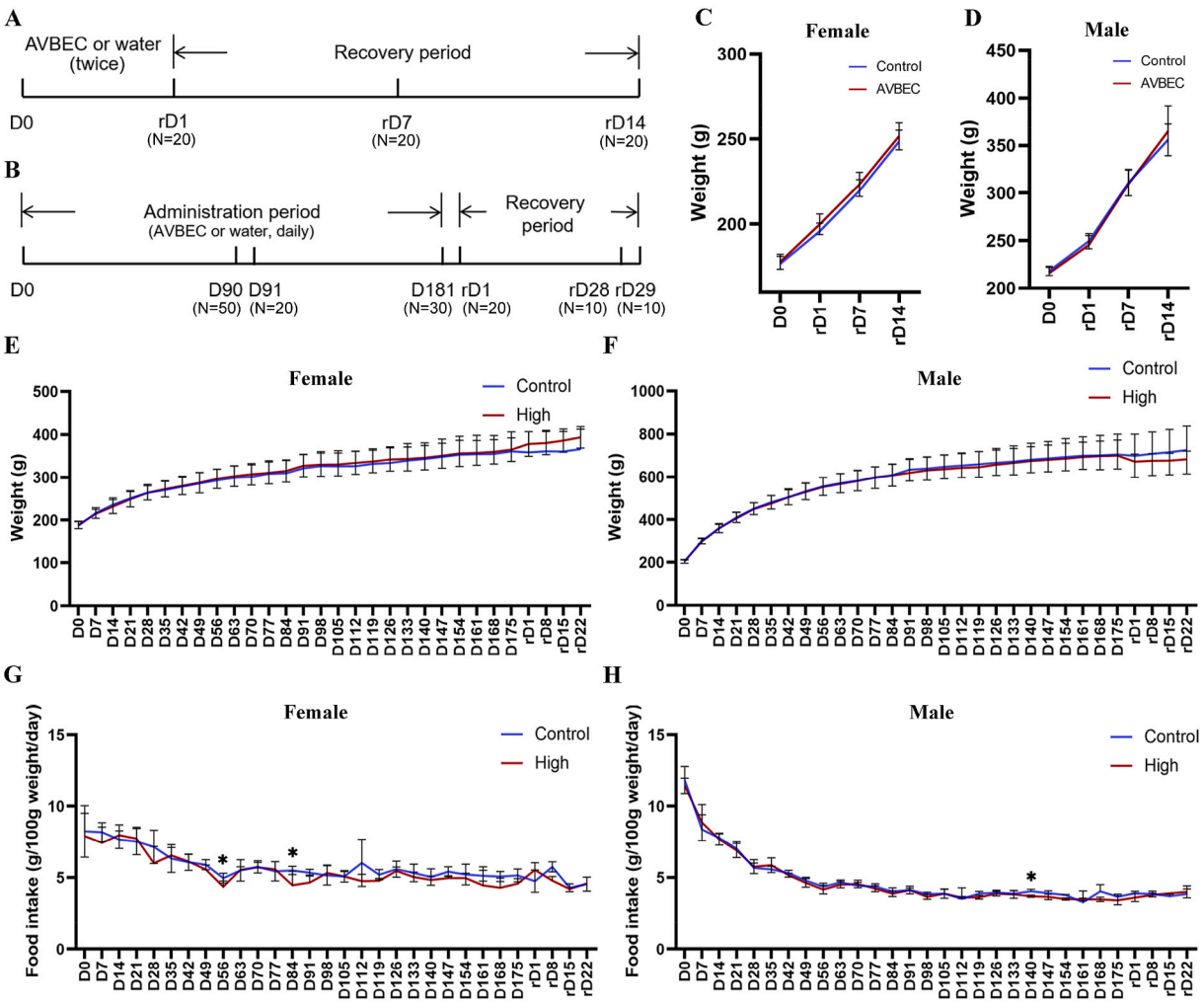

Fig. 2. Body weight and food consumption of SD rats in acute and chronic toxicity studies. (A) Timeline of the acute toxicity study. (B) Timeline of the 6-month chronic toxicity study. Weights of female rats (C) and male rats (D) in the acute toxicity study. Weights of female rats (E) and male rats (F) in the 6-month chronic toxicity study. Food consumption of female rats (G) and male rats (H) in the 6-month chronic toxicity study. Statistical analysis was performed using the T-test.

Based on the analysis of organ histology, we further performed the clinical pathological observations. From the clinical blood chemistry analysis conducted, at the end of the recovery period, the Urea concentrations of the AVBEC-treated groups were found to be lower than that in the negative control group (Table 1 and Table S3). This finding is considered to be not toxicologically significant because no increases in CRE, ALT and AST were observed at the end of the recovery period (Table 1 and Table S3). In addition, there was no biological significance with a decrease of AST in the males from high-dose group since it is not observed in other periods, especially in the recovery groups. Additionally, the TP of males from the high-dose group was lower than that of the negative control group, which may be resulted from the loss of urine protein (Tables 1 and 2).

The urinalysis results showed that the concentration of PRO in rats from medium- and high-dose groups was higher than in the control group, which was partly accompanied by an increase in blood Urea, and there was no abnormality at the end of the recovery period (Table 2 and Table S4, Table 1 and Table S3). This was considered to be relative to the substance and result in changes with other indicators in urinalysis. From Table 2, BIL of the high-dose group was higher than that of the control groups, which is considered to be unrelated to AVBEC because there was no significant difference between the two groups with respect to ALT and TBIL levels during the same period (Table 1). Similarly, the higher level of KET is considered to be unrelated to AVBEC, as Glu from blood and urine in the AVBEC-treated group was within the normal range (Table 2). In the recovery group, the changes in WBC were regarded to be non-biological and non-toxic significance because the relative indicators were also within the normal range. Furthermore, no significant changes in BLO and SG were observed in the recovery groups. During the administration period, the alterations in PH in rats from the AVBEC-treated groups were not dose-dependent and time-dependent (Table 2 and Table S4). In summary, there is no biological and toxicological significance in the clinical blood chemistry and urinalysis in both sexes from the high-dose group.

3.2.2.3. Hematology. Peripheral blood analysis showed that the significant changes in BASO and RET were not dose-dependent or time-dependent (Table 3 and Table S5). There were no correlative findings of RBC and HGB related to the decrease in MCV at the end of the treatment, which can be considered to be non-toxicological significance. From the coagulation assay, there was a significant decrease in the PT of rats from the high-dose group during the administration period, whereas this was not observed in the recovery group. Most importantly, according to the bone marrow cytology, there was no abnormality in the bone marrow of rats in each dose group. The specimens of the high-dose group were similar to those of the control group, which showed similar active proliferation of cells, and the cell morphology, size, and proportion of each stage were normal. Moreover, mature red blood cells are

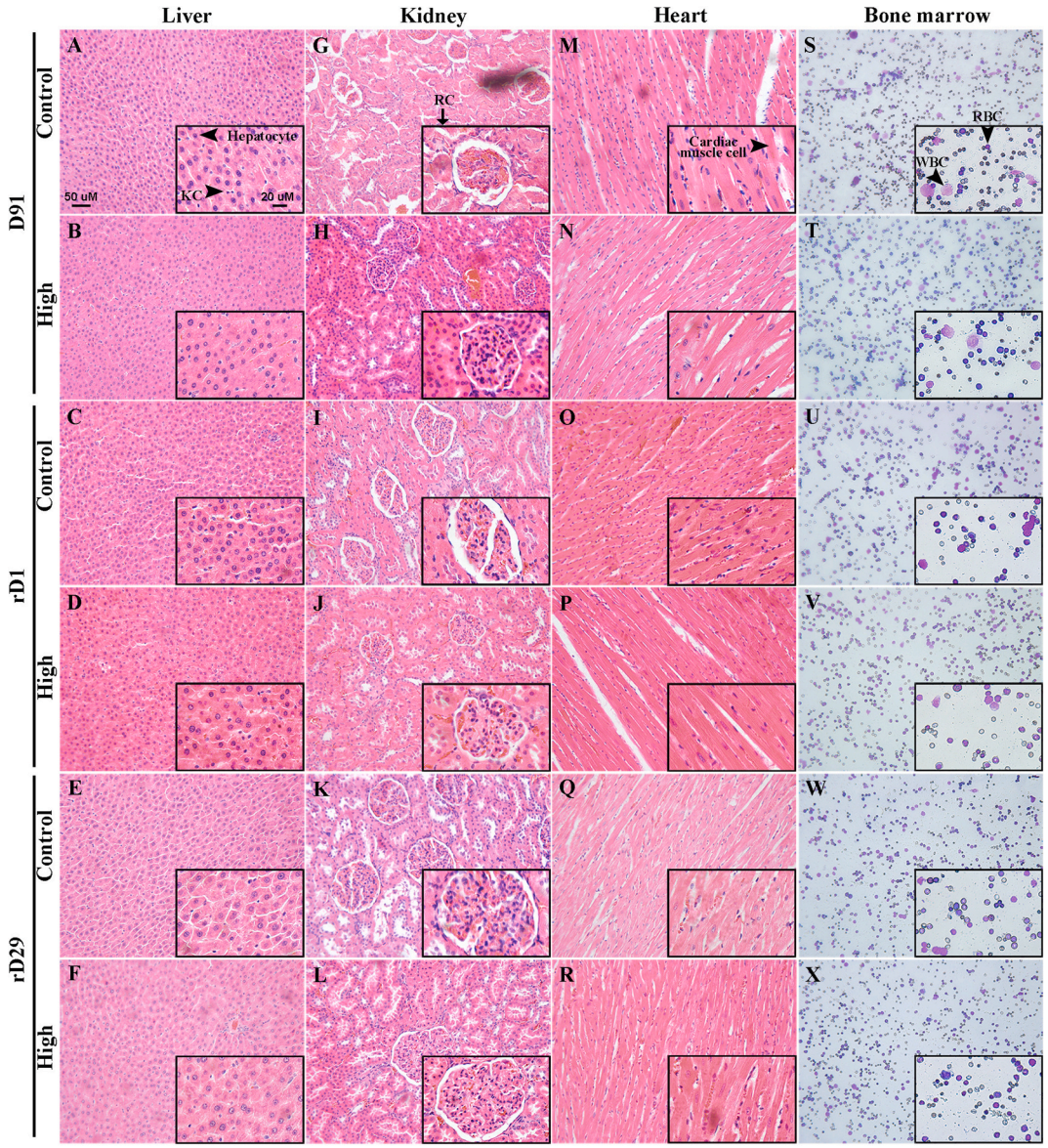

Fig. 3. Organ specimens from organs in the 6-month chronic toxicity study. The histopathological analysis (H&E staining, × 200 and × 400) of liver (A–F), kidney (G–L), and heart (M–R) of the negative control group and high-dose group were performed at D91, rD1, and rD29, respectively. (S–X) The bone marrow smears (× 200 and × 400) of the negative control group and high-dose group were performed at D91, rD1, and rD29, respectively. The scale bar is shown in A. KC: Kupffer cell; RC: renal corpuscle; WBC: white blood cell; RED: red blood cell.

double-concave and disc-shaped, and uniform in size (Fig. 3S–X).

Consistent with the results of organ chemistry, AVBEC did not cause organ toxicity nor induce changes in crucial organ structures or chemical function; moreover, no peripheral and central immune system damage was observed, suggesting that AVBEC is sufficiently safe for further functional analysis.

3.3. Anticancer potency of AVBEC in human cell lines

With the safety evaluation of AVBEC from acute and chronic toxicity studies, we explored the function of AVBEC in human cell lines, including malignant breast cancer (MCF-7 and MDA-MB-231), lung cancer (NCI-H 524 and NCI-H 1975) cells and non-cancer cell line (HEK293).

3.3.1. AVBEC induced cell apoptosis

First, the CCK-8 assay was employed to investigate the effect of AVBEC on cell viability, including MCF-7, MDA-MB-231, NCI-H 524 and NCI-H 1975 cells. After the treatment with AVBEC at various concentrations for 24 h, cell viability was found to be significantly lower than that of the control group in a dose-dependent manner (Fig. S1A-D). We further carried out propidium iodide (PI) and annexin V (FITC) double staining to detect apoptotic cells. Treatment with 10 mg/ml AVBEC for 8 h significantly increased the apoptosis of MCF-7 cells (Fig. S1E, H and U), MDA-MB-231 (Fig. S1I, L and V), NCI-H 524 (Fig. S1M, P and W) and NCI-H 1975 (Fig. S1Q, T and X), and the lower concentrations of AVBEC, 2.5 mg/ml and 5 mg/ml induced cell apoptosis in MCF-7 cells (Fig. S1E-G, U) and NCI-H 524 cells (Fig. S1M-O, W). Time-course analyses indicated that the treatment with 10 mg/ml AVBEC could induce apoptosis of MCF-7 (Fig. 4A, B and Q), NCI-H 524 (Fig. 4I, J and S) and NCI-H 1975 (Fig. 4M, N and T) cells within 4 h, and MDA-MB-231 cells within 8 h (Fig. 4E, G and R). In response to previous results, western blotting showed increased levels of cleaved caspase-3 in the four cell lines treated with 10 mg/ml AVBEC (Fig. 4U–X), confirming that the apoptotic pathways were activated.

Additionally, we explore the specificity of AVBEC to induce cell

Table 1
Blood biochemistry analysis of rats treated with AVBEC.

Parameter	D91(N = 10)		rD1(N = 10)		rD29(N = 5)	
	Control	High	Control	High	Control	High
Female						
ALT (U/L)	26.9 ± 6.7	30.0 ± 3.5	40.9 ± 19.7	39.2 ± 14.8	52.9 ± 21.2	38.0 ± 6.7
CRE(μmol/L)	39.6 ± 8.8	37.8 ± 7.3	44.9 ± 14.6	40.6 ± 7.4	42.9 ± 8.3	35.5 ± 5.3
GLU (mmol/L)	7.74 ± 0.63	7.24 ± 0.90	7.65 ± 0.40	7.97 ± 0.71	7.65 ± 1.05	8.16 ± 0.73
Urea (mmol/L)	7.38 ± 1.42	7.01 ± 1.04	8.05 ± 2.45	6.50 ± 1.04	8.50 ± 1.19	**5.79 ± 0.45**[a]
TBIL (μmol/L)	0.9 ± 0.5	0.8 ± 0.3	1.9 ± 0.4	1.7 ± 0.5	1.7 ± 0.7	2.0 ± 0.6
ALP(U/L)	51.7 ± 10.7	49.7 ± 11.7	33.9 ± 6.3	33.5 ± 8.6	29.6 ± 4.2	42.8 ± 13.9
CHOL (mmol/L)	2.06 ± 0.44	2.15 ± 0.24	2.32 ± 0.48	2.21 ± 0.36	1.90 ± 0.26	2.18 ± 0.48
GGT (U/L)	−0.32 ± 0.54	−1.03 ± 2.10	−0.06 ± 0.42	0.04 ± 0.53	0.15 ± 0.37	0.05 ± 0.25
TP (g/L)	67.48 ± 2.33	65.83 ± 3.10	70.13 ± 5.68	68.09 ± 1.84	65.54 ± 3.42	71.46 ± 3.20
ALB (g/L)	46.94 ± 1.42	45.57 ± 2.49	47.63 ± 3.95	45.07 ± 2.46	45.00 ± 2.06	48.85 ± 1.61
CK(U/L)	451.7 ± 146.6	397.7 ± 71.8	243.6 ± 67.3	321.6 ± 99.7	505.0 ± 64.2	356.9 ± 95.9
TG (mmol/L)	0.76 ± 0.50	0.62 ± 0.31	1.30 ± 0.89	0.69 ± 0.28	0.99 ± 0.50	1.64 ± 0.55
AST (U/L)	112.0 ± 25.4	105.9 ± 17.2	133.6 ± 76.9	118.8 ± 36.4	185.9 ± 71.8	105.6 ± 35.5
Na^+(mmol/L)	141.2 ± 1.6	140.6 ± 1.3	140.5 ± 0.9	139.9 ± 1.0	140.4 ± 1.3	141.4 ± 0.7
K^+(mmol/L)	4.5 ± 0.4	4.5 ± 0.4	4.2 ± 0.2	4.5 ± 0.4	4.2 ± 0.4	3.8 ± 0.1
Cl^-(mmol/L)	109.0 ± 2.2	109.5 ± 2.1	109.4 ± 1.4	109.0 ± 0.8	109.6 ± 1.8	109.2 ± 2.2
Male						
ALT (U/L)	30.2 ± 5.5	27.5 ± 8.1	34.8 ± 15.0	35.5 ± 24.1	94.4 ± 120.1	26.5 ± 4.4
CRE(μmol/L)	32.8 ± 3.7	35.5 ± 9.5	31.7 ± 4.5	31.5 ± 6.6	37.5 ± 8.1	29.9 ± 2.2
GLU (mmol/L)	7.32 ± 0.58	7.73 ± 1.46	8.47 ± 1.01	8.33 ± 1.30	8.32 ± 1.01	8.15 ± 1.25
Urea (mmol/L)	7.70 ± 1.06	6.70 ± 1.27	5.97 ± 0.91	5.52 ± 1.12	8.05 ± 1.73	**6.10 ± 1.03**[a]
TBIL (μmol/L)	1.0 ± 0.4	1.2 ± 0.2	1.6 ± 0.6	1.8 ± 0.7	2.6 ± 0.8	1.5 ± 0.2
ALP(U/L)	87.5 ± 15.1	97.7 ± 16.0	71.0 ± 11.1	65.3 ± 8.6	103.2 ± 39.7	68.5 ± 7.4
CHOL (mmol/L)	2.06 ± 0.46	1.98 ± 0.26	2.00 ± 0.32	2.04 ± 0.44	1.82 ± 0.41	1.92 ± 0.37
GGT (U/L)	−0.40 ± 0.31	−0.15 ± 0.30	0.30 ± 0.41	0.51 ± 0.60	0.32 ± 0.58	0.20 ± 0.19
TP (g/L)	57.35 ± 2.15	**59.88 ± 1.99**[a]	61.12 ± 1.55	61.54 ± 1.19	59.88 ± 3.22	62.16 ± 2.07
ALB (g/L)	39.41 ± 1.95	40.92 ± 1.38	39.22 ± 1.23	39.34 ± 1.06	39.13 ± 1.71	39.99 ± 1.81
CK(U/L)	451.0 ± 69.0	371.9 ± 111.1	385.9 ± 179.3	297.0 ± 122.6	427.1 ± 165.2	447.4 ± 234.6
TG (mmol/L)	0.53 ± 0.16	0.59 ± 0.25	0.80 ± 0.16	0.91 ± 0.67	0.90 ± 0.33	0.98 ± 0.32
AST (U/L)	131.5 ± 22.1	**108.3 ± 14.7**[a]	125.0 ± 34.1	121.6 ± 29.3	156.6 ± 97.4	107.5 ± 24.8
Na^+(mmol/L)	140.0 ± 0.8	140.0 ± 0.8	140.2 ± 0.7	140.7 ± 1.3	140.5 ± 0.9	140.7 ± 0.9
K^+(mmol/L)	4.7 ± 0.2	4.8 ± 0.2	4.6 ± 0.2	4.7 ± 0.2	4.5 ± 0.2	4.5 ± 0.3
Cl^-(mmol/L)	107.9 ± 1.2	107.3 ± 1.3	108.2 ± 0.7	108.0 ± 1.2	108.9 ± 1.1	109.7 ± 0.9

[a] Represent the significance level for the difference with the Control for $p < 0.05$.

Table 2
Urinalysis of rats treated with AVBEC.

Parameter	D90(N = 25)		D181(N = 15)		rD28(N = 5)	
	Control	High	Control	High	Control	High
Female						
GLU	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
BIL	1.2 ± 0.4	1.2 ± 0.4	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.0	1.4 ± 0.5
KET	1.2 ± 0.5	1.3 ± 0.6	1.0 ± 0.0	1.2 ± 0.4	1.0 ± 0.0	1.2 ± 0.4
SG	1.023 ± 0.007	1.027 ± 0.005	1.021 ± 0.007	1.023 ± 0.007	1.029 ± 0.002	1.024 ± 0.005
BLO	1.4 ± 0.8	1.2 ± 0.4	1.5 ± 0.8	1.1 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
pH	7.9 ± 1.0	**6.8 ± 0.7**[a]	7.3 ± 1.1	7.4 ± 0.7	6.7 ± 0.4	**8.6 ± 0.2**[a]
PRO	1.9 ± 1.2	**2.6 ± 1.2**[a]	1.9 ± 1.3	1.7 ± 1.1	1.8 ± 1.1	2.6 ± 1.5
URO	4.2 ± 3.5	3.7 ± 2.6	1.0 ± 0.0	1.0 ± 0.0	8.3 ± 7.0	5.8 ± 5.7
NIT	1.0 ± 0.2	1.1 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
WBC	1.2 ± 0.8	1.0 ± 0.0	1.4 ± 0.5	**1.1 ± 0.3**[a]	1.0 ± 0.0	**1.6 ± 0.5**[a]
Male						
GLU	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
BIL	1.0 ± 0.0	**1.8 ± 0.4**[a]	1.1 ± 0.3	**1.6 ± 0.5**[a]	1.2 ± 0.4	1.2 ± 0.4
KET	2.1 ± 1.0	**2.8 ± 0.8**[a]	1.5 ± 0.7	**2.5 ± 0.9**[a]	2.4 ± 0.9	1.8 ± 0.8
SG	1.023 ± 0.007	1.026 ± 0.007	1.018 ± 0.006	**1.029 ± 0.002**[a]	1.026 ± 0.004	1.028 ± 0.004
BLO	2.0 ± 0.7	**1.5 ± 0.7**[a]	2.5 ± 0.8	**1.7 ± 0.7**[a]	2.6 ± 0.9	2.0 ± 1.0
pH	7.7 ± 1.0	7.3 ± 0.9	7.5 ± 1.0	7.1 ± 0.7	7.3 ± 0.9	7.7 ± 0.6
PRO	3.5 ± 1.0	**4.0 ± 0.9**[a]	3.1 ± 1.0	**4.0 ± 0.5**[a]	3.4 ± 1.3	4.0 ± 1.7
URO	3.7 ± 2.6	6.3 ± 5.6	1.0 ± 0.0	1.1 ± 0.4	5.8 ± 5.7	5.8 ± 5.7
NIT	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
WBC	1.4 ± 0.5	**1.0 ± 0.0**[a]	1.7 ± 0.6	1.3 ± 0.5	2.4 ± 0.5	2.4 ± 0.9

[a] Represent the significance level for the difference with the Control for $p < 0.05$.

apoptosis in non-cancer cell line (HEK293). And we found that 16 h treatment with 10 mg/ml AVBEC induced HEK293 cells apoptosis (3% more cells than control) and the ratio of apoptotic cell was significantly lower than that of cancer cells (Fig. 5A–D).

3.3.2. *Investigation of specificity of AVBEC*

3.3.2.1. Investigation of malic acid in cell lines. The safety evaluation revealed that there was no toxicity in rats with AVBEC although it was found to induce cancer cell apoptosis, suggesting that AVBEC may work with specific components or specific targets. It has been reported that malic acid has an anti-proliferative effect in HaCaT cells through the inhibition of cell cycle progression at G0/G1, and the induction of cell apoptosis through endoplasmic reticulum (ER) stress- and mitochondria-dependent pathways (Zhang et al., 2017). Herein we explored the effect of malic acid on breast and lung cancer cells at a corresponding concentration of 3.6 mg/ml. Apoptosis and necrosis analysis showed that malic acid induced cell necrosis time-dependently in MCF-7 (Fig. 6A, B and Q), MDA-MB-231 (Fig. 6E, F and R), NCI-H 524 (Fig. 6I, J and S) and NCI-H 1975 (Fig. 6M, N and T). Compared with malic acid treatment in a certain duration, AVBEC did not induce cell

Table 3
Hematologic analysis of rats treated with AVBEC.

Parameter	D91(N = 10)		rD1(N = 10)		rD29(N = 5)	
	Control	High	Control	High	Control	High
Female						
WBC(× 10^9/L)	3.08 ± 1.38	3.81 ± 1.51	2.73 ± 0.91	3.05 ± 1.75	3.26 ± 1.66	3.63 ± 0.89
RBC(× 10^{12}/L)	8.02 ± 0.43	8.16 ± 0.28	7.84 ± 0.37	8.16 ± 0.40	7.95 ± 0.19	8.03 ± 0.27
HGB (g/dL)	14.4 ± 0.6	14.5 ± 0.6	14.3 ± 0.7	14.3 ± 0.8	14.2 ± 0.5	14.5 ± 0.3
HCT (%)	42.6 ± 1.7	43.4 ± 1.5	45.1 ± 2.2	45.5 ± 2.6	45.9 ± 1.6	46.6 ± 0.5
MCV(fL)	53.2 ± 2.0	53.2 ± 1.6	57.6 ± 1.6	**55.8 ± 1.6**[a]	57.7 ± 2.4	58.1 ± 1.8
MCH(pg)	18.0 ± 0.9	17.8 ± 0.6	18.2 ± 0.8	17.5 ± 0.5	18.0 ± 0.9	18.0 ± 0.5
MCHC(g/dL)	33.7 ± 0.7	33.4 ± 0.5	31.7 ± 0.8	31.4 ± 0.4	31.1 ± 0.6	31.0 ± 0.3
PLT (× 10^9/L)	1043.3 ± 87.7	1049.7 ± 101.6	956.2 ± 81.8	1035.8 ± 112.1	1084.2 ± 114.8	959.0 ± 86.1
NEUT (%)	14.1 ± 5.3	17.6 ± 4.0	20.8 ± 2.9	22.1 ± 6.9	15.4 ± 5.5	21.5 ± 5.4
LYM(%)	81.6 ± 6.1	76.9 ± 5.8	73.2 ± 3.6	72.1 ± 7.4	78.6 ± 6.4	72.1 ± 6.3
MONO(%)	2.0 ± 0.8	2.7 ± 0.8	2.8 ± 1.0	2.7 ± 1.1	3.0 ± 0.7	2.9 ± 0.8
EOS(%)	2.2 ± 0.8	2.8 ± 1.8	3.0 ± 0.9	2.9 ± 0.9	2.9 ± 0.6	3.3 ± 1.1
BASO(%)	0.0 ± 0.0	**0.1 ± 0.1**[a]	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0
Ret (%)	2.7 ± 0.4	2.5 ± 0.4	2.3 ± 0.5	1.9 ± 0.6	2.0 ± 0.3	1.9 ± 0.2
PT (Sec)	8.9 ± 0.2	**8.4 ± 0.3**[a]	8.9 ± 0.5	8.7 ± 0.1	9.3 ± 0.3	9.0 ± 0.1
APTT (Sec)	16.6 ± 1.2	15.3 ± 0.5	15.6 ± 1.5	15.0 ± 1.0	15.6 ± 1.2	14.5 ± 0.5
Male						
WBC(× 10^9/L)	7.23 ± 2.19	6.54 ± 3.46	6.36 ± 1.48	5.95 ± 1.52	6.77 ± 2.18	6.31 ± 1.94
RBC(× 10^{12}/L)	9.04 ± 0.27	9.03 ± 0.37	9.18 ± 0.41	9.07 ± 0.22	8.94 ± 0.32	8.95 ± 0.31
HGB (g/dL)	15.1 ± 0.5	15.2 ± 0.6	15.2 ± 0.4	14.8 ± 0.5	14.8 ± 0.6	14.6 ± 0.5
HCT (%)	45.2 ± 1.5	45.9 ± 2.4	48.6 ± 2.0	48.1 ± 1.7	48.4 ± 1.3	48.2 ± 1.7
MCV(fL)	50.0 ± 1.0	50.8 ± 1.3	53.0 ± 1.7	53.0 ± 1.9	54.2 ± 2.0	54.0 ± 2.6
MCH(pg)	16.7 ± 0.5	16.9 ± 0.3	16.6 ± 0.6	16.3 ± 0.5	16.6 ± 0.7	16.3 ± 0.8
MCHC(g/dL)	33.3 ± 0.8	33.2 ± 0.7	31.3 ± 0.8	30.8 ± 0.4	30.6 ± 0.4	30.2 ± 0.2
PLT (×10^9/L)	1082.2 ± 68.9	1088.1 ± 171.8	1083.5 ± 111.9	1092.7 ± 203.2	1076.6 ± 78.2	1124.8 ± 184.3
NEUT (%)	19.8 ± 4.5	23.0 ± 8.6	22.4 ± 5.3	24.2 ± 4.8	26.8 ± 3.4	28.0 ± 3.8
LYM(%)	75.0 ± 4.8	71.5 ± 9.0	72.0 ± 5.7	71.1 ± 4.8	67.4 ± 3.9	66.0 ± 3.6
MONO(%)	3.2 ± 0.7	3.2 ± 0.8	3.3 ± 1.7	2.6 ± 0.8	3.2 ± 1.2	3.7 ± 1.1
EOS(%)	2.0 ± 0.6	2.2 ± 1.4	2.2 ± 0.5	1.9 ± 0.2	2.5 ± 0.7	2.2 ± 0.8
BASO(%)	0.0 ± 0.0	0.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0
Ret (%)	2.2 ± 0.2	**2.6 ± 0.2**[a]	2.3 ± 0.3	2.4 ± 0.4	2.1 ± 0.5	2.1 ± 0.4
PT (Sec)	10.2 ± 0.8	9.3 ± 0.6	10.2 ± 0.7	**9.6 ± 0.4**[a]	10.2 ± 0.6	10.0 ± 0.2
APTT (Sec)	20.9 ± 2.3	19.8 ± 1.9	17.8 ± 1.2	17.2 ± 1.9	17.6 ± 1.7	18.1 ± 1.0

[a] Represent the significance level for the difference with the Control for $p < 0.05$.

apoptosis and necrosis in MCF-7 (Fig. 6C, D and Q), MDA-MB-231 (Fig. 6G, H and R), and NCI-H 1975 (Fig. 6O, P and T), and induced less cell apoptosis and necrosis in NCI-H 524 cells (Fig. 6K, L and S).

Similarly, 3.6 mg/ml malic acid induced HEK293 cell necrosis and AVBEC did not induce HEK293 cell apoptosis and necrosis in a certain duration (Fig. 5E–I). Considering that we previously observed that 16 h treatment with 10 mg/ml AVBEC induced HEK293 cell apoptosis with a significantly lower ratio than cancer cells (Fig. 5A–D), this suggested that malic acid is toxic to cancer cells and non-cancer cells and that the toxicity of malic acid was reduced with the integrated AVBEC compound. In addition, it is noteworthy that, unlike malic acid is toxic to cancer cells and non-cancer cells, AVBEC could induce cancer cells apoptosis with a specific way from non-cancer cells.

3.3.2.2. AVBEC modulated mitochondrial function. As shown in Table S1, most of the dominant components of AVBEC from HPLC-MS/MS are metabolites that work in the tricarboxylic acid cycle (TCA) of mitochondria. Mitochondria are important bioenergetic organelles that play an essential role in signaling pathways, including metabolism and cell apoptosis. We examined the function of AVBEC in mitochondria and found that the fluorescence intensity of ROS was significantly enhanced with a shift to right compared with the control (Fig. 7A–E). Additionally, the ATP concentration in cell lysates decreased when cells were treated with AVBEC for 8 h (Fig. 7F).

4. Discussion

Aloe vera is widely used as a functional food and therapeutic product. It is more used as a folk medicine and limited official clinical medicine typically applied as a laxative. Thus, it warrants controlled and comprehensive studies to investigate the adverse effects, toxicity and efficacy of *Aloe vera*. There have been plenty of reports researching on the safety evaluations of *Aloe vera in vivo* and *in vitro*. Diverse toxic and adverse effects have been identified, which might be relevant to the tested parts of the plant, the processes of extraction or purification, indicated dosages and the tested animal system (Cosmetic Ingredient Review Expert, 2007; Guo and Mei, 2016). In the current study, we performed acute and chronic toxicity test on 240 rats in total according to the CFDA Guidelines (CFDA, 20140513), and no mortality was observed. Moreover, clinical testing and histological observation revealed that AVBEC did not give rise to any organ toxicity or immune system damage in tested rats (Figs. 2 and 3, Tables 1–3 and S2-5), and we found that treatment with 10 mg/ml AVBEC decreased the cell population of S phase in MCF-7, MDA-MB-231, NCI-H 524 and NCI-H 1975 (data not shown), which suggested that AVBEC induced cells in S phase to undergo apoptosis while without toxicity in the rats immune system. Thus, AVBEC may exert effects on cellular function with specific components or targets.

Many TCM compounds are considered to have low or almost no toxicity because the phytochemicals from herbs function synergistically and reduce the toxicity of individual ingredients (Hsiao and Liu, 2010; Lao et al., 2012). And, it is well believed that bioactive compounds from Aloe vera function synergistically instead of single chemical component. Fifty compounds were detected in AVBEC obtained by HPLC-MS/MS, and malic acid was found to be the most dominant organic acid (Table S1). Unlike the purchased malic acid treatment which time-dependently induced cell necrosis in cancer cells and normal cells, AVBEC did not exhibit similar effects according to the results of the present study (Figs. 5 and 6). This observation may result from the synergistic function produced within the AVBEC compound or the presence of some antidotal components from AVBEC, for instance, another dominant component, lactobionic acid (LA, Table S1). LA offers perfectly efficient targeted drug delivery and this is regarded as a more potent approach for effective, precise and safe therapeutic interventions (Alonso et al., 2013; Fu et al., 2014). It warrants investigation of

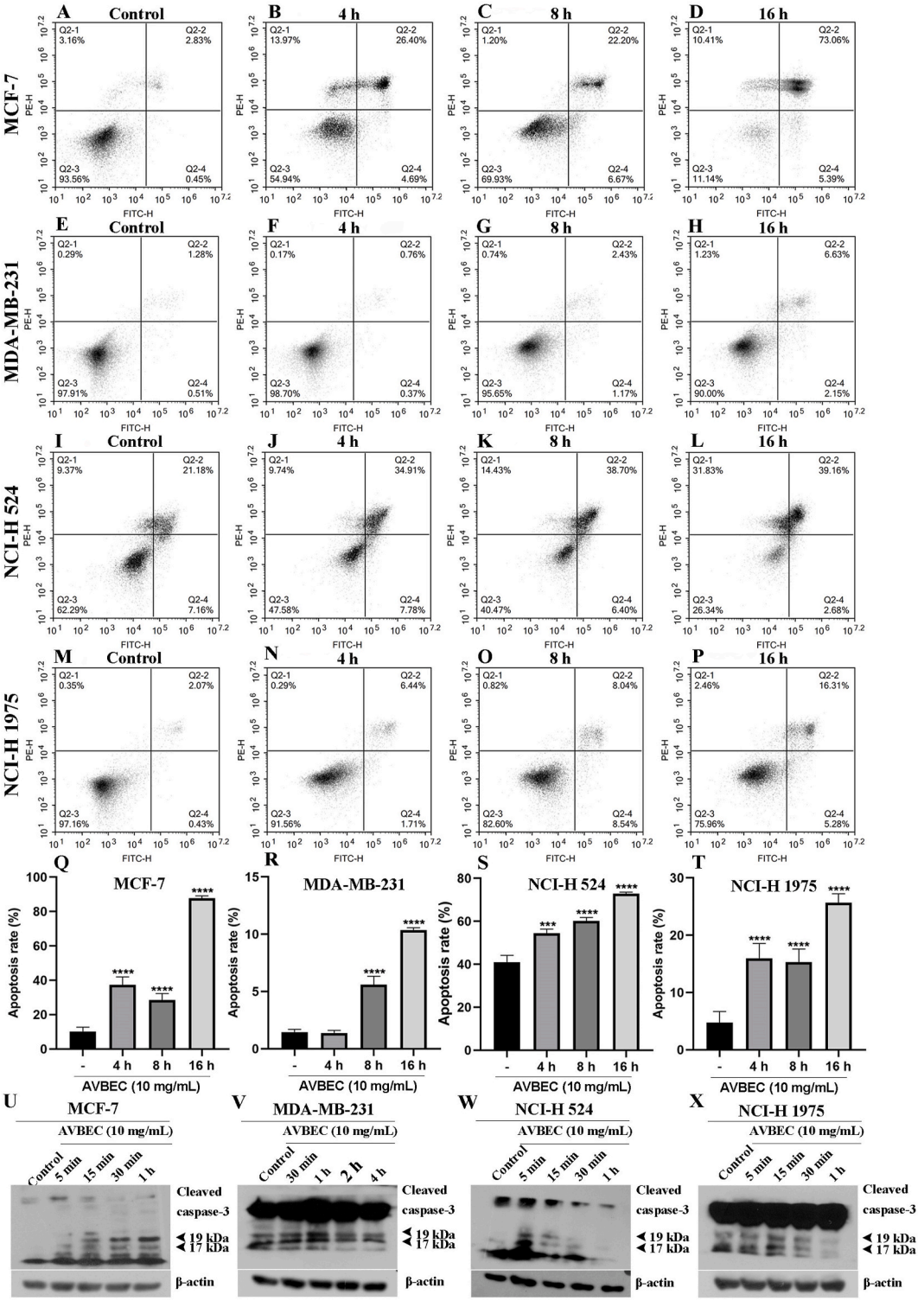

Fig. 4. AVBEC induced breast and lung cancer cells apoptosis in a time-dependent manner. (A–D) MCF-7, (E–H) MDA-MB-231, (I–L) NCI-H 524, and (M–P) NCI-H 1975 were treated with AVBEC (10 mg/ml) for 4 h, 8 h and 16 h. The apoptotic cells were detected by FITC-PI. (Q–T) Apoptosis ratios are expressed as mean ± SD. (U–X) Expressions of cleaved caspase-3 were detected by Western blot after treatment with AVBEC (10 mg/ml) for varying durations. The experiments were repeated for three times independently. Statistical analysis was performed with one-way ANOVA. *, **, *** and **** represent the significance levels for the difference with the control for $p < 0.05$, 0.01, 0.001 and 0.0001, respectively.

components in AVBEC and we will further explore its function with purified or specific components from AVBEC.

Moreover, the toxicity of drugs can be significantly reduced by increasing their specificity. Our data show that AVBEC induces apoptosis by targeting the mitochondria (Fig. 7). Apoptosis is an efficient way to prevent tumor progression because defects in the apoptotic pathway play an essential role in the transformation from normal cells to dysfunctional cells and evasion from programmed cell death (Wong, 2011). Most cell death is managed by mitochondria, and this organelle is known as the center for maintaining the precise balance

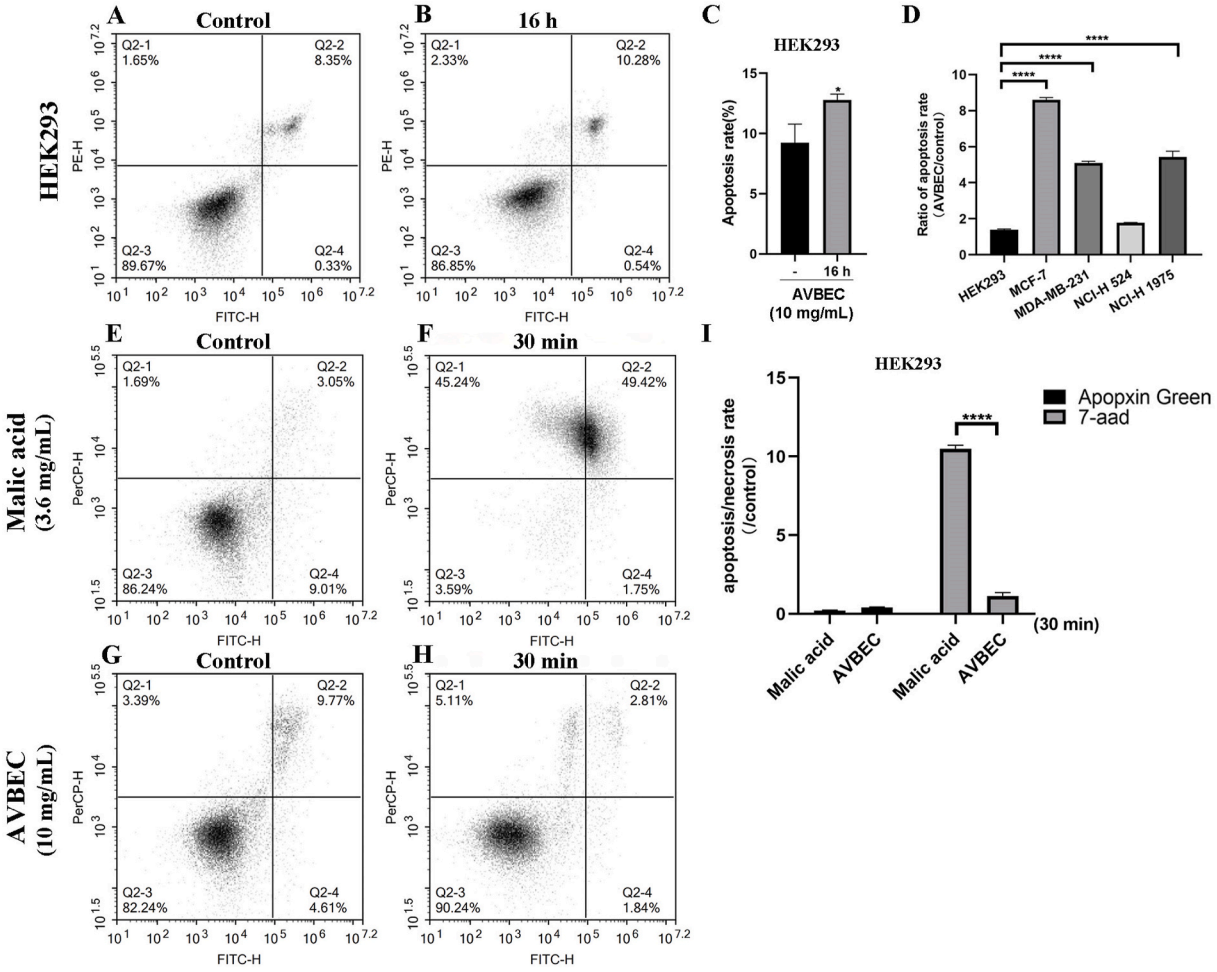

Fig. 5. Investigation of AVBEC and malic acid in HEK293 cell. (A–B) HEK293 were treated with AVBEC (10 mg/ml) for 16 h and the apoptotic cells were detected by FITC-PI. (C) Apoptosis ratios of HEK293 were expressed as mean ± SD. (D) Apoptotic cells ratios were normalized against the control and are expressed as mean ± SD. (E–H) HEK293 were treated with malic acid (3.6 mg/ml) and AVBEC (10 mg/ml) for 30 min, and the rate of apoptosis and necrosis were detected. (I) Ratios of apoptosis and necrosis were normalized against control and are expressed as mean ± SD. The experiments were repeated for three times independently. Statistical analysis was performed with T-test and one-way ANOVA. *, **, *** and **** represent the significance levels for the difference with the control for $p < 0.05$, 0.01, 0.001 and 0.0001, respectively.

of multiple signaling pathways, including energy production, biosynthesis, redox homeostasis, calcium and cell fate regulation. However, many cancer cells prefer aerobic glycolysis (Warburg effect) bypassing the TCA cycle with oxidative phosphorylation and electron transport with damaged mitochondria or uncoupling of mitochondria (Vander Heiden et al., 2009). Under these conditions, mitochondrial recoupling has become a novel strategy for cancer therapy since the aberrant or malignant cells with damaged mitochondria are prone to undergo apoptosis (Baffy et al., 2011). Indeed, increased production of ROS could induce cell apoptosis mediated by mitochondria, p53, ER stress or MAPK (Redza-Dutordoir and Averill-Bates, 2016). Simultaneously, ROS overproduction impairs mitochondrial function and the ATP production (Thirupathi and de Souza, 2017). Previous studies have suggested that aloe-emodin can induce mitochondria-mediated glioma cell apoptosis (Ismail et al., 2013) and lung cancer cell apoptosis by targeting ROS generation (Wu et al., 2017). In the current study, we observed an increase of ROS and decrease in ATP following AVBEC treatment in breast and lung cancer cells as well as the activation of the energy sensor, AMPK from breast cancer cells, MCF-7 and MDA-MB-231 (data not shown). Activation of AMPK directly represents the energy status of cells and regulates numerous cellular activities, including cell cycle and cell apoptosis, most through phosphorylation of P53 (Nieminen et al., 2013). We also found the increased protein levels of Bax and enhanced Cyto-C when treated with AVBEC in two lung cancer cell lines (data not shown). In summary, increased cellular ROS and decreased ATP production due to the treatment of AVBEC suggested the influence of AVBEC on mitochondrial function, and the mechanism by which mitochondria play in mediating cancer cell apoptosis with AVBEC treatment is worthy of further study.

The main reason for the adverse effects of chemotherapy is the low or non-selectivity of anticancer agents, which arises because they are designed to kill cells with higher proliferation rates, including cancer cells and immune cells residing in the bone marrow. We observed AVBEC induced the cell apoptosis to a considerably different degree as the number for apoptotic cancer cells was far greater than that of HEK293 cells (Figs. 4 and 5A-D). As a transformed cell line, HEK293 cells can easily and quickly reproduce with abnormal chromosome and protein expression, which might explain the phenomenon that the designed normal cells were also partially subject to death by AVBEC (Thomas and Smart, 2005). Considering the evaluation of AVBEC did not show any influence on the peripheral and central hemopoietic/immune system, it shows a significant potential as a natural anticancer medicine with high efficacy and safety.

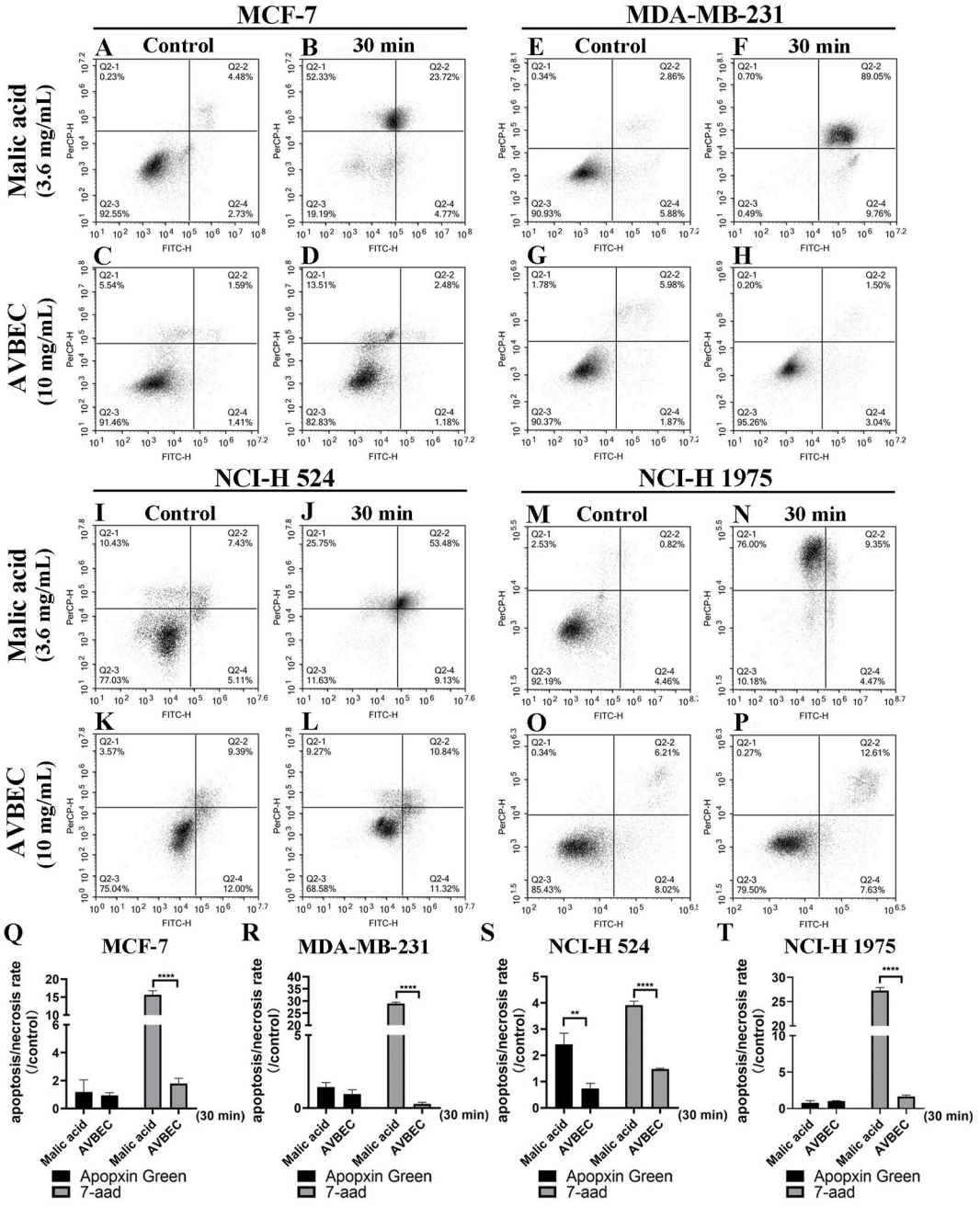

Fig. 6. Investigation of malic acid in breast and lung cancer cells. (A–D) MCF-7, (E–H) MDA-MB-231, (I–L) NCI-H 524, and (M–P) NCI-H 1975 were treated with malic acid (3.6 mg/ml) and AVBEC (10 mg/ml) for 30 min, and the apoptosis and necrosis were detected. (Q–T) Ratios of apoptosis and necrosis were normalized against the control and are expressed as mean ± SD. The experiments were repeated for three times independently. Statistical analysis was performed with T-test. *, **, *** and **** represent the significance levels for the difference with the control for $p < 0.05$, 0.01, 0.001 and 0.0001, respectively.

5. Conclusion

We found that AVBEC did not yield toxicity in rats from acute and chronic safety evaluation; moreover, it was found to induce cancer cell apoptosis, which may be because AVBEC modulates the function of mitochondria. Components within AVBEC work synergistically to detoxify some toxic components and finally facilitate their function. As an integrated compound, AVBEC induced cell apoptosis by targeting mitochondria *in vitro* and did not cause toxic changes in the main organs or to the peripheral and central immune system *in vivo*, suggesting that it could be a potential natural medicine for anticancer treatment with excellent safety and efficacy.

Authors' contributions

This work was carried out in collaboration between all authors: Conceived and designed the experiments: JJL and XCY. Performed the experiments: XLT, DL, JMC, CQL, WJX and MJZ. Analyzed the data: JJL, XCY, XLT, ML and DL. Wrote the paper: JJL, XCY, XLT and JXD. All authors have read and approved the manuscript.

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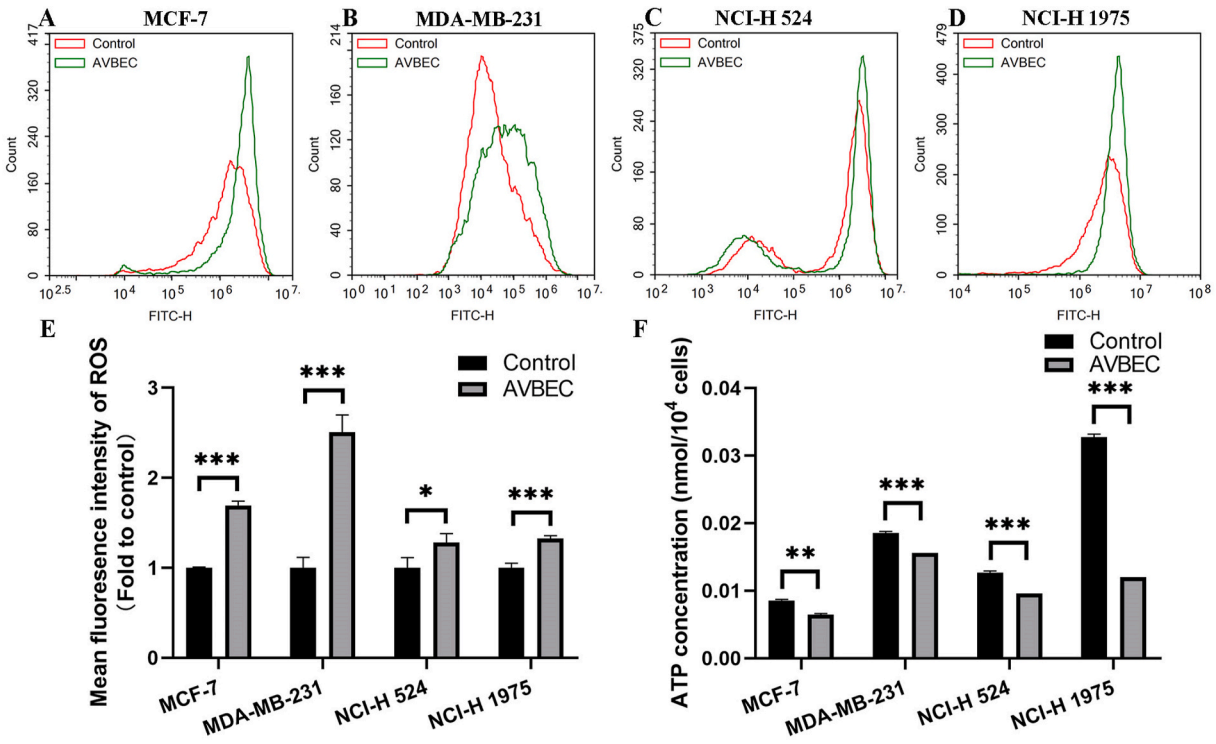

Fig. 7. AVBEC modulated mitochondrial function. (A–D) AVBEC (10 mg/ml) treated four cell lines (MCF-7, MDA-MB-231, NCI-H 524, and NCI-H 1975) for 8 h and the intracellular ROS was detected by DCFH-DA. (E) The mean fluorescence intensity of ROS was expressed as mean ± SD. (F) AVBEC (10 mg/ml) treated four cell lines for 8 h and ATP concentration were detected. The data was showed as mean ± SD. The experiments were repeated for three times independently. Statistical analysis was performed with T-test. *, **, *** and **** represent the significance levels for the difference with the control for $p < 0.05$, 0.01, 0.001 and 0.0001, respectively.

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CRediT authorship contribution statement

Xueli Tong: performing the experiments, Formal analysis, Writing – original draft. **Min Li:** Formal analysis. **Di Li:** performing the experiments, Formal analysis. **Chunqin Lao:** performing the experiments. **Jingmian Chen:** performing the experiments. **Weijie Xu:** performing the experiments. **Junxi Du:** Writing – original draft, Writing – review & editing. **Meijiao Zhang:** performing the experiments. **Xiangcai Yang:** Formal analysis, experiment design, Writing – original draft. **Jiejing Li:** Formal analysis, experiment design, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2021.114434.

References

Alonso, S., Rendueles, M., Diaz, M., 2013. Bio-production of lactobionic acid: current status, applications and future prospects. Biotechnol. Adv. 31 (8), 1275–1291.
Baffy, G., Derdak, Z., Robson, S.C., 2011. Mitochondrial recoupling: a novel therapeutic strategy for cancer? Br. J. Canc. 105 (4), 469–474.
Budai, M.M., Varga, A., Milesz, S., Tőzsér, J., Benkő, S., 2013. Aloe vera downregulates LPS-induced inflammatory cytokine production and expression of NLRP3 inflammasome in human macrophages. Mol. Immunol. 56 (4), 471–479.
Cholongitas, E., Katsoudas, S., Spyros, K., Dourakis, S., Spyros, D., 2005. Henoch-Schonlein purpura associated with Aloe vera administration. Eur. J. Intern. Med. 16 (1), 59–60.
Cosmetic Ingredient Review Expert, P., 2007. Final report on the safety assessment of AloeAndongensis extract, aloe andongensis leaf Juice,aloe arborescens leaf extract, aloe arborescens leaf juice, aloe arborescens leaf protoplasts, aloe barbadensis flower extract, aloe barbadensis leaf, aloe barbadensis leaf extract, aloe barbadensis leaf Juice,aloe barbadensis leaf polysaccharides, aloe barbadensis leaf water, aloe ferox leaf extract, aloe ferox leaf juice, and aloe ferox leaf juice extract. Int. J. Toxicol. 26 (Suppl. 2), 1–50.
Damani, M.R., Shah Ar Fau - Karp, C.L., Karp Cl Fau - Orlin, S.E., Orlin, S.E., 2015. Treatment of ocular surface squamous neoplasia with topical Aloe vera drops. Case Rep. 34 (1), 87–89.
Diehl, K.H., Hull R Fau - Morton, D., Morton D Fau - Pfister, R., Pfister R Fau - Rabemampianina, Y., Rabemampianina Y Fau - Smith, D., Smith D Fau - Vidal, J.M., Vidal Jm Fau - van de Vorstenbosch, C., van de Vorstenbosch, C., 2001. A good practice guide to the administration of substances and removal of blood, including routes and volumes. J. Appl. Toxicol. 21 (1), 15–23.
Farrugia, C.-J.E., Burke, E.S., Haley, M.E., Bedi, K.T., Gandhi, M.A., 2019. The use of aloe vera in cancer radiation: an updated comprehensive review. Compl. Ther. Clin. Pract. 35, 126–130.
Ferreira, M., Teixeira, M., Silva, E., Selores, M., 2007. Allergic contact dermatitis to Aloe vera. Contact Dermatitis 57 (4), 278–279.
Fu, F., Wu, Y., Zhu, J., Wen, S., Shen, M., Shi, X., 2014. Multifunctional lactobionic acid-modified dendrimers for targeted drug delivery to liver cancer cells: investigating the role played by PEG spacer. ACS Appl. Mater. Interfaces 6 (18), 16416–16425.
Gorkom, B.V., Vries, E.D., 1999. Anthranoid laxatives and their potential carcinogenic effects. Aliment. Pharmacol. Ther. 13 (4), 443–452.
Guo, X., Mei, N., 2016. Aloe vera: a review of toxicity and adverse clinical effects. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 34 (2), 77–96.
Haddad, P., Amouzgar-Hashemi, F., Samsami, S., Chinichian, S., Oghabian, M.A., 2013. Aloe vera for prevention of radiation-induced dermatitis: a self-controlled clinical trial. Curr. Oncol. 20 (4), e345–348.
Hamiza, O.O., Rehman, M.U., Khan, R., Tahir, M., Khan, A.Q., Lateef, A., Sultana, S., 2013. Chemopreventive effects of aloin against 1,2-dimethylhydrazine-induced preneoplastic lesions in the colon of Wistar rats. Hum. Exp. Toxicol. 33 (2), 148–163.
Harlev, E., Nevo, E., Lansky, E.P., Ofir, R., Bishayee, A., 2012. Anticancer potential of aloes: antioxidant, antiproliferative, and immunostimulatory attributes. Planta Med. 78 (9), 843–852.

Hekmatpou, D., Mehrabi, F., Rahzani, K., Aminiyan, A., 2019. The effect of aloe vera clinical trials on prevention and healing of skin wound: a systematic review. Iran. J. Med. Sci. 44 (1), 1–9.
Hsiao, W.L., Liu, L., 2010. The role of traditional Chinese herbal medicines in cancer therapy–from TCM theory to mechanistic insights. Planta Med. 76 (11), 1118–1131.
Huseini, H.F., Kianbakht, S., Hajiaghaee, R., Dabaghian, F.H., 2012. Anti-hyperglycemic and anti-hypercholesterolemic effects of Aloe vera leaf gel in hyperlipidemic type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. Planta Med. 78 (4), 311–316.
Hussain, A., Sharma, C., Khan, S., Shah, K., Haque, S., 2015. Aloe vera inhibits proliferation of human breast and cervical cancer cells and acts synergistically with cisplatin. Asian Pac. J. Cancer Prev. APJCP 16 (7), 2939–2946.
Im, S.-A., Kim, J.-W., Kim, H.-S., Park, C.-S., Shin, E., Do, S.-G., Park, Y.I., Lee, C.-K., 2016. Prevention of azoxymethane/dextran sodium sulfate-induced mouse colon carcinogenesis by processed Aloe vera gel. Int. Immunopharm. 40, 428–435.
Ismail, S., Haris, K., Abdul Ghani, A.R.I., Abdullah, J.M., Johan, M.F., Mohamed Yusoff, A.A., 2013. Enhanced induction of cell cycle arrest and apoptosis via the mitochondrial membrane potential disruption in human U87 malignant glioma cells by aloe emodin. J. Asian Nat. Prod. Res. 15 (9), 1003–1012.
Jiao, P., Jia, Q., Fau - Randel, G., Randel G Fau - Diehl, B., Diehl B Fau - Weaver, S., Weaver S Fau - Milligan, G., Milligan, G., 2010. Quantitative 1H-NMR spectrometry method for quality control of Aloe vera products. J. AOAC Int. 93 (3), 642–848.
Kumar, S., Tiku, A.B., 2015. Immunomodulatory potential of acemannan (polysaccharide fromAloe vera) against radiation induced mortality in Swiss albino mice. Food Agric. Immunol. 27 (1), 72–86.
Lao, L., Xu, L., Xu, S., 2012. Traditional Chinese medicine. Integr. Pediatr. Oncol. 125–135.
Lee, J., Lee, M.S., Nam, K.W., 2014a. Acute toxic hepatitis caused by an aloe vera preparation in a young patient: a case report with a literature review. Korean J. Gastroenterol. 64 (1), 54–58.
Lee, M.-C., Liao, J.-D., Huang, W.-L., Jiang, F.-Y., Jheng, Y.-Z., Jin, Y.-Y., Tseng, Y.-S., 2014b. Aloin-induced cell growth arrest, cell apoptosis, and autophagy in human non-small cell lung cancer cells. Biomark. Genomic Med. 6 (4), 144–149.
Li, P., Wu, H., Wang, Y., Peng, W., Su, W., 2020. Toxicological evaluation of naringin: acute, subchronic, and chronic toxicity in Beagle dogs. Regul. Toxicol. Pharmacol. 111, 104580.
Lin, N.-H., Yang, H.-W., Su, Y.-J., Chang, C.-W., 2019. Herb induced liver injury after using herbal medicine: a systemic review and case-control study. Medicine 98 (13), e14992.
Liu, C., Cui, Y., Pi, F., Cheng, Y., Guo, Y., Qian, H., 2019. Extraction, purification, structural characteristics, biological activities and pharmacological applications of acemannan, a polysaccharide from aloe vera: a review. Molecules 24 (8), 1554.
Lopez, Z., Femenia, A., Nunez-Jinez, G., Salazar Zuniga, M.N., Cano, M.E., Espino, T., Knauth, P., 2019. In vitro immunomodulatory effect of food supplement from aloe vera. Evid. Based Complement Alternat. Med. 2019, 5961742.
López, Z., Núñez-Jinez, G., Avalos-Navarro, G., Rivera, G., Salazar-Flores, J., Ramírez, J. A., Ayil-Gutiérrez, B.A., Knauth, P., 2017. Antioxidant and cytotoxicological effects of aloe vera food supplements. J. Food Qual. 1–10.
Majumder, R., Das, C.K., Mandal, M., 2019. Lead bioactive compounds of Aloe vera as potential anticancer agent. Pharmacol. Res. 148, 104416.
Mansouri, P., Haghighi, M., Beheshtipour, N., Ramzi, M., 2016. The effect of aloe vera solution on chemotherapy-induced stomatitis in clients with lymphoma and leukemia: a randomized controlled clinical trial. Int. J. Commun. Base. Nurs. Midwifery 4 (2), 119–126.
Morrow, D.M., Rapaport, M.J., Strick, R.A., 1980. Hypersensitivity to aloe. Arch. Dermatol. 116 (9), 1064–1065.
Nieminen, A.I., Eskelinen, V.M., Haikala, H.M., Tervonen, T.A., Yan, Y., Partanen, J.I., Klefstrom, J., 2013. Myc-induced AMPK-phospho p53 pathway activates Bak to sensitize mitochondrial apoptosis. Proc. Natl. Acad. Sci. U. S. A. 110 (20), E1839–E1848.
Parlati, L., Voican, C.S., Perlemuter, K., Perlemuter, G., 2017. Aloe vera-induced acute liver injury: a case report and literature review. Clin. Res. Hepatol. Gastroenterol. 41 (4), e39–e42.
Radha, M.H., Laxmipriya, N.P., 2015. Evaluation of biological properties and clinical effectiveness of Aloe vera: a systematic review. J. Tradit. Complement Med. 5 (1), 21–26.
Rajasekaran, S., Sivagnanam, K., Fau - Subramanian, S., Subramanian, S., 2005. Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacogn. Res. 575 (1), 90–96.
Rathor, N., Mehta, A.K., Sharma, A.K., Mediratta, P.K., Sharma, K.K., 2012. Acute effect of Aloe vera gel extract on experimental models of pain. Inflammation 35 (6), 1900–1903.
Redza-Dutordoir, M., Averill-Bates, D.A., 2016. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim. Biophys. Acta 1863 (12), 2977–2992.
Sahebnasagh, A., Ghasemi, A., Akbari, J., Alipour, A., Lashkardoost, H., Ala, S., Salehifar, E., 2017. Successful treatment of acute radiation proctitis with aloe vera: a preliminary randomized controlled clinical trial. J. Alternative Compl. Med. 23 (11), 858–865.
Sanders, B., Ray, A.M., Goldberg, S., Clark, T., McDaniel, H.R., Atlas, S.E., Farooqi, A., Konefal, J., Lages, L.C., Lopez, J., Rasul, A., Tiozzo, E., Woolger, J.M., Lewis, J.E., 2017. Anti-cancer effects of aloe-emodin: a systematic review. J. Clin. Transl. Res. 3 (3), 283–296.
Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A., Bray, F., 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J. Clin. 71 (3), 209–249.
Thirupathi, A., de Souza, C.T., 2017. Multi-regulatory network of ROS: the interconnection of ROS, PGC-1 alpha, and AMPK-SIRT1 during exercise. J. Physiol. Biochem. 73 (4), 487–494.
Thomas, P., Smart, T.G., 2005. HEK293 cell line: a vehicle for the expression of recombinant proteins. J. Pharmacol. Toxicol. Methods 51 (3), 187–200.
Vander Heiden, M.G., Cantley, L.C., Thompson, C.B., 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324 (5930), 1029–1033.
Wong, R.S.Y., 2011. Apoptosis in cancer: from pathogenesis to treatment. J. Exp. Clin. Canc. Res. 30 (1), 87.
Wu, Y.-Y., Zhang, J.-H., Gao, J.-H., Li, Y.-S., 2017. Aloe-emodin (AE) nanoparticles suppresses proliferation and induces apoptosis in human lung squamous carcinoma via ROS generation in vitro and in vivo. Biochem. Biophys. Res. Commun. 490 (3), 601–607.
Yang, H.N., Kim, D.J., Kim, Y.M., Kim, B.H., Sohn, K.M., Choi, M.J., Choi, Y.H., 2010. Aloe-induced toxic hepatitis. J. Kor. Med. Sci. 25 (3), 492–495.
Zagórska-Dziok, M., Furman-Toczek, D., Dudra-Jastrzębska, M., Zygo, K., Stanisławek, A., Kapka-Skrzypczak, L., 2017. Evaluation of clinical effectiveness of Aloe vera – a review. J. Pre-Clin. Clin. Res. 11 (1), 86–93.
Zhang, B., Tornmalm, J., Widengren, J., Vakifahmetoglu-Norberg, H., Norberg, E., 2017. Characterization of the role of the malate dehydrogenases to lung tumor cell survival. J. Canc. 8 (11), 2088–2096.