Antitumor Properties and Modulation of Antioxidant Enzymes' Activity by *Aloe vera* Leaf Active Principles Isolated *via* Supercritical Carbon Dioxide Extraction

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Abstract: The aim of this study was to evaluate the potential anticancer properties and modulatory effect of selected *Aloe vera* (*A. vera*) active principles on antioxidant enzyme activities. Thus, three anthraquinones (Namely: aloesin, aloe-emodin and barbaloin) were extracted from *A. vera* leaves by supercritical fluid extraction and subsequently purified by high performance liquid chromatography. Additionally, the N-terminal octapeptide derived from verectin, a biologically active 14 kDa glycoprotein present in *A. vera*, was also tested. *In vivo*, active principles exhibited significant prolongation of the life span of tumor-transplanted animals in the following order: barbaloin> octapeptide> aloesin > aloe-emodin. *A. vera* active principles exhibited significant inhibition on Ehrlich ascite carcinoma cell (EACC) number, when compared to positive control group, in the following order: barbaloin> aloe-emodin > octapeptide > aloesin. Moreover, in trypan blue cell viability assay, active principles showed a significant concentration-dependent cytotoxicity against acute myeloid leukemia (AML) and acute lymphocytes leukemia (ALL) cancerous cells. Furthermore, in MTT cell viability test, aloe-emodin was found to be active against two human colon cancer cell lines (i.e. DLD-1 and HT2), with IC_{50} values of 8.94 and 10.78 μM, respectively. Treatments of human AML leukemic cells with active principles (100 μg ml^{-1}) resulted in varying intensities of internucleosomal DNA fragmentation, hallmark of cells undergoing apoptosis, in the following order: aloe-emodin> aloesin> barbaloin> octapeptide. Intererstingly, treatment of EACC tumors with active principles resulted in a significant elevation activity of key antioxidant enzymes (SOD, GST, tGPx, and LDH). Our data suggest that the tested *A. vera* compounds may exert their chemo-preventive effect through modulating antioxidant and detoxification enzyme activity levels, as they are one of the indicators of tumorigenesis. These findings are discussed in the light of the potential of *A. vera* plant extracts for developing efficient, specific and non-toxic anticancer drugs that are affordable for developing countries.

Keywords: Natural products, *Aloe vera*, Anticancer, Antioxidant enzymes, Acute myeloid leukemia, Acute lymphocytes leukemia, Ehrlich ascite carcinoma cells.

1. INTRODUCTION

Aloe barbadensis Miller (*Aloe vera*), a member of the *Liliaceae* family, is a perennial succulent with turgid lace-shaped green leaves, and is referred to as the miracle/ healing plant or the silent healer. *Aloe vera* possesses confirmed versatile curative or healing actions. Worldwide, a total of 360 *A. vera* species (commonly accepted as *A. vera*) are cultivated. It has been long used as an ethnomedicine and is currently being utilized in a variety of commercial products, because of its unique therapeutic properties [1]. Particularly, the whole gel extract of *A. vera* has been reported to have various pharmacologic properties, specifically to promote wound, burn and frost-bite healing, in addition to having anti-inflammatory, immunomodulatory and gastroprotective properties [2]. Moreover, some specially prepared *A. vera* extracts possess many biological activities such as hypoglycemic, hypolipidemic, antifungal, anticancer, antioxidant and immunoprotective [3-7]. Many attempts have been made to isolate single, biologically active components, to examine their effects, and clarify their functional mechanism. Many active components have been isolated from *A. vera* and studied for their biological activities. These active ingredients primarily include glycoproteins, anthraquinones, polysaccharides and low–molecular-weight species [1].

Pharmacologically active anthraquinones present in *A. vera* include aloe-emodin, barbaloin and aloesin (Fig. **1**) [1]. Various biological activities in high molecular weight material have been ascribed to polysaccharide fractions in *A. vera* gel [7,8]. An example of biologically active glycoproteins, isolated from *A. vera* gel, is verectin; a 14 kDa lectin with cell proliferation-promoting activity on hamster and human dermal fibroblasts, and prominent free radical scavenging activity against superoxide anion, generated by the xanthine/xanthine oxidase system, as well as inhibition of cy-

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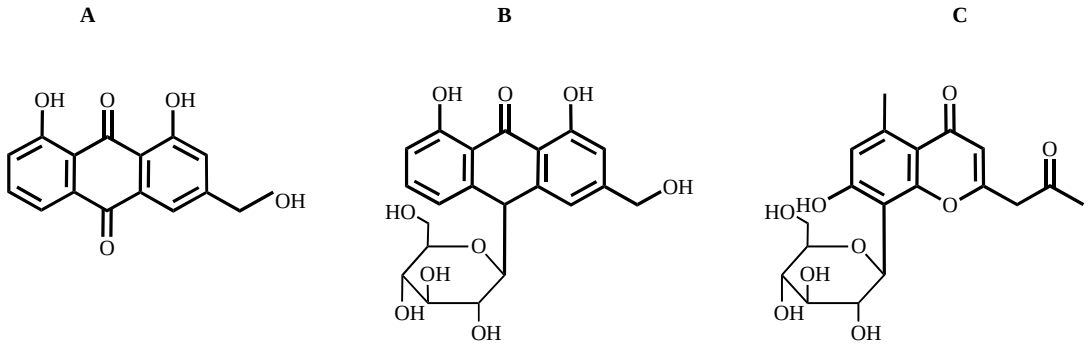

Fig. (1). Chemical structure of active principles isolated from *Aloe vera* leaves. (**A**) Aloe-emodin (1,8-dihydroxy-3-hydroxymethyl-9,10-anthracenedione), (**B**) Barbaloin (10-Glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10H)-anthracenone) and (**C**) Aloesin (7-hydroxy-5-methy-2-(2-oxopropyl)-8-(glucopyranosyl)chromen-4-one).

clooxygenase-2 and reduction of thromboxane A_2 synthase level *in vitro* [9,10].

Evidence from epidemiological and experimental studies has indicated that certain naturally occurring phytochemical phenolic compounds (e.g. alkaloids and flavonoids), particularly those ingested in the human diet, exhibit antimutagenic anticarcinogenic properties, and play a beneficial role in the prevention of certain types of cancer [11-15]. Aloe-emodin and aloesin are major plant phenolic compounds which are known to possess several pharmacological effects such as antimicrobial, anti-inflammatory, hepatoprotective and antimutagenic [1]. Moreover, *A. vera* gel extracts have previously been shown to exhibit antioxidant activity by virtue of polysaccharides content and its potent free radical-scavenging activity [8].

In this context, antioxidant enzyme markers (e.g. Superoxide dismutase, SOD; total glutathione peroxidase, tGpx; glutathione S-transferase, GST; and lactase dehydrogenase, LDH) have previously been measured in the serum of Ehrlich ascite carcinoma cells (EACC) administered animals. It is well documented that during carcinogenesis a significant alteration of serum stimulated GST, level, extent of GPx, SOD and LDH activity, is observed [16]. Moreover, the inter-relationship and role of these key enzymes, in characterizing the progression of tumor, have successfully been established in various reports [16]. However, our current knowledge on the protective mode of action of *A. vera* active principles in carcinogenesis and the underlying mechanism has remained largely unknown.

The isolation and/or purification of *A. vera* active principles have attracted much attention. Traditionally, liquid solvent extraction with organic solvents is the most currently applied technique [17]. However, these techniques involve the use of large amount of toxic solvents which have to be eliminated before analysis, in addition to the long extraction time and the complex procedures. Beside these difficulties liquid solvent extraction still suffers from the disadvantage of oxidative transformation during the solvent removal [18]. Therefore, supercritical fluid extraction (SFE) with carbon dioxide is a good alternative to conventional liquid solvent extraction approaches, especially in the case of plant materials because of elimination of the use of environmentally hazardous solvents, the simple and fast extraction technique applied and fairly clean extract obtained [19,20]. Carbon dioxide has been used almost exclusively as the extraction medium, due to its inertness and non-toxic properties [21].

Oxidative stress is caused by any imbalance between the production of reactive oxygen species (ROS) and the activation of antioxidant systems. Oxidative damage has long been implicated in the process of carcinogenesis as well as the degree of malignant transformation of most types of tumors [22-24]. Carcinogenesis is a multistep process where ROS were found to enhance carcinogenesis at all stages: initiation, promotion, and progression.

The current investigation employed an optimized protocol for the isolation of active principles from *A. vera* based on supercritical carbon dioxide fluid extraction [20]. A detailed characterization of *in vivo* anticancer activity of the isolated *A. vera* active principles (i.e. aloe-emodin, barbaloin, aloesin and octapeptide) on survival/life span, body weight and tumor cell number of EACC-transplanted mice was carried out. Moreover, the *in vitro* cytotoxic activity of these four active principles was tested, against five distinct human and animal cancer cell lines, by trypan blue and MTT cell viability assays. Furthermore, the capacity of the compounds to induce apoptosis-mediated cell death was examined against human AML cell lines. Since insufficient capacity of protective antioxidant system can result in cancer, another objective of the current study was to examine the activity of key antioxidant enzymes (i.e. SOD, GST, GPx, LDH) in EACC tumor cells, and the modulatory effect of these active principles on their activities.

2. MATERIALS AND METHODS

2.1. Chemicals

Fetal bovine serum, $_{L}$-glutamine, penicillin and streptomycine were purchased from Gibco BRL (Grand Island NY, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis MO, USA), and were of analytical grade. HPLC grade methanol, acetonitrile, phosphoric acid as well as methanol and ethyl acetate for liquid solvent extraction were purchased from BDH Laboratory supplies, England. Authentic samples of aloe-emodin and barbaloin were kindly donated by Placenta-Aloe Research Institute, Japan. The verectin-derived N-terminal octapeptide (DEDNVLLT) was synthesized by Peptide Institute, Japan.

2.2. Plant Materials

The leaves of *A. vera* plants, cultivated in the garden of college of Pharmacy, Tanta University, Egypt, were collected and identified by Prof. Hassan El Kady, Dept. of Botany, Faculty of Science, Tanta University. Specimen of *A. vera* was available for inspection at the herbal garden, Faculty of Pharmacy, Tanta University, Egypt. The leaves were cut longitudinally and the gel inside was removed. The outer part of the leaves were cut to small pieces and dried at 40 °C in the oven. The dried leaves were thoroughly grinded in a domestic mixer.

2.3. Supercritical Carbon Dioxide Fluid Extraction

Supercritical fluid extraction (SFE) was essentially performed according to the method previously described [20], with some modifications. Briefly, SFE was performed on ISCO SFX 2-10 super-critical fluid extractor, model 260D using carbon dioxide in a constant flow rate at 0.3 ml min^{-1}. The temperature was controlled by a column oven adjusted between 40 and 70 °C. The pressure in the system was regulated from 2700 to 7000 *Psi*. In each experiment, 4 g of the dried plant powder were placed in the extraction cell. The static extraction time was kept at 15 min during the whole experiment. The dynamic extraction time was controlled between 10 and 60 min. For the measurements of aloe-emodin and barbaloin extraction, the extracts were filtered through a 0.22 μm membrane filter (Gelman, England) and the volumes were adjusted to 25 ml with methanol prior to HPLC analysis. Quantitative HPLC analysis was performed on LC1445 system organizer, Eppendorf Biotronic using a C18 column (250× 406 mm i.d, 5 μ Hypersil) operated under isocratic conditions and applying a mobile phase consisting of 25% phosphoric acid (0.1% in water) in acetonitrile. The flow rate was adjusted at 1 ml min^{-1}. Aloe-emodin and barbaloin identification was carried out by retention time co-chromatography and spiking with pure aloe-emodin while their quantitative determination was done using a standard curve. Triplicate injections were made from every sample and the average of the peak areas was used to quantify aloe-emodin and barbaloin concentration. Aloe-emodin and barbaloin were detected at 290 nm using an UV/visible detector.

2.4. Prepration and Direct Sequencing of N-Terminal Octapeptide in Verectin

A novel procedure for peptide sequencing was employed for the identification of N-terminal octapeptide derived from verectin [25]. Briefly, verectin (0.3 mM) was first subjected to 12% SDS-PAGE analysis. After digestion by lysylendopeptidase enzyme, the peptides were separated by 12% SDS-PAGE and then electro-blotted onto a PVDF membrane. After CBBR-250 staining, the peptide bands were excised from the membrane sheet. The N-terminal amino acid sequence was determined using a Hewlett-Packard G 1005A automated protein sequencing system calibrated with phenylthiohydantoin (PTH)-amino acid standards, prior to each sequencing run, by the Edman degradation method. The PVDF membrane blotted sample was located directly into the sequencer. Peptide separation by HPLC was applied to the N-terminal amino acid sequence as previously described [25].

2.5. Determination of Cell Viability by Trypan Blue Assay

Cytotoxicity of active principles was tested against two different types of human cancer cells namely: acute lymphocytes leukemia (ALL) and acute myeloid leukemia (AML). Additionally, Ehrlich ascite carcinoma cells (EACC) was employed as a representative of animal tumor cell lines. ALL and AML cells were harvested from adult leukemia patients or healthy relatives, aged 18–65 years that were admitted to the National Cancer Institute, Cairo University. International protocols governing the ethical treatment of patients were followed. In addition, animals were transplanted with EACC from an immortal culture obtained from National Cancer Institute, Cairo University, and maintained at mice transplanted line. International protocols governing the ethical treatment of animals were followed. Separation and preparation of ALL and AML mononuclear cell layers were carried out as previously reported [26]. The AML, ALL and EACC cell counts were adjusted to 10^5 cells/0.1 ml (counting both mature and immature cells). The cytotoxicity of each active principal against AML, ALL and EACC cells was determined by the trypan blue exclusion test [27]. The cell counts were adjusted to (1×10^5 cell/ 0.1 ml). Next, 0.1 ml of the cell suspension containing 10^5 cells was added to each of four 1.8 ml screw-caps sterile eppendorf tubes. Four concentrations of active principles was added (50, 100, 150 and 200 μg ml^{-1}), three replicas each. For each concentration one tube served as negative control, where culture medium was added instead of the active principal. The tubes were incubated at 37 °C in the presence of 5% (v/v) CO_2 for 24 h (dark condition, humidified air). After overnight incubation, cells were stained with trypan blue (0.2 %) dissolved in PBS, and the number viable (unstained) versus dead (stained) cells was estimated. Two hundred cells were counted for EACC tube.

2.6. Determination of Cell Viability by MTT Assay

Human colon cell lines DLD-1 and HT-29, frequently used for screening potential anticancer agents, were obtained from the Institute of Cancer Therapeutics (Bradford University, UK). The cytotoxicity of aloe-emodin and barbaloin against human colon cell lines DLD-1 and HT-29 was determined by the MTT {3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide} assay [28]. All cells were routinely maintained as monolayer cultures in RPMI 1640 medium with HEPES buffer (25 mM) supplemented with foetal calf serum (10%), sodium pyruvate (1 mM), L-Glutamine (2 mM) and penicillin/streptomycin (50 IU ml^{-1}/ 50 μg ml^{-1}). Briefly, 1 x 10^4 cells ml^{-1} (180 μl) were inoculated into each well of a 96 well plate and incubated overnight at 37 °C in a humidified incubator (5% CO_2). Both aloe-emodin and barbaloin were dissolved in DMSO and diluted in culture medium to provide a broad range of concentrations; the maximum DMSO concentration in any well was 0.1%. Medium was removed from each well and replaced with drug solutions (8 wells per drug concentration) and incubated at 37 °C for 4 days before the assessment of chemosensitivity. Culture medium was replaced with fresh medium (180 μl) prior to the addition of 20 μl of MTT solution (0.5 mg ml^{-1}). Treatment of living cells with MTT produces a dark blue formazan product, whereas no such staining is observed in dead

cells. Following 4 h incubation at 37 °C, medium plus MTT was removed from each well, and formazan crystals were dissolved in DMSO (150 µl/well). The absorbance of the resulting solutions was determined at 550 nm using a Labsystems Multiskan Plus Microplate reader. Results were expressed in terms of percentage survival taking the mean absorbance of control samples to be 100 % cell survival (Labsystems Genesis V3.04 software). Cytotoxicity was expressed as IC_{50} value (i.e. the concentration of drug required to produce 50 % inhibition of growth). 5-Fluorouracil, which is commonly used in the treatment of colon cancer, was used as a positive control. All experiments were performed in triplicate.

2.7. DNA Fragmentation Assay

DNA fragmentation was assayed as previously described [29]. DNA was extracted from mature (normal cells) and leukemic immature AML white blood cells before and after treatment with the *A. vera* active principles (100 µg ml^{-1}). Cells were washed with PBS, lysed in 400 µl of ice-cold lysis buffer (50 mM Tris pH 7.4, 20 mM EDTA, 0.5% Triton X-100) for 30 min, and then centrifuged. RNase (100 µg ml^{-1}) was added to the supernatant, which was then incubated at 50 °C for 30 min, followed by the addition of 200 µg ml^{-1} proteinase K and further incubation at 37 °C for 1 h. Fragmented DNA was extracted with phenol:chloroform: isoamyl aclochol (25:24:1, v/v/v) and precipitated at -20 °C with cold 100% ethanol:sodium acetate (0.3 M) (2v:1v). The DNA fragments were electrophoresed on a 2.0% agarose gel containing 0.1 mg ml^{-1} ethidium bromide.

2.8. Animals and Experimental Design

A total of 60 healthy male Swiss albino mice (7–8 week-old), weighing between 20-25 g each, fed on normal diet were used throughout the study. Mice were maintained under controlled environment conditions of temperature and humidity with alternating 12 h light/dark cycles. All animals were fed standard pellet diet (Gold Mohor rat feed, Ms. Hindustan Lever Ltd, Mumbai) and water *ad libitum*. For carcinogenesis, animals were transplanted with EACC from an immortal culture obtained from National Cancer Institute, Cairo University, and maintained at mice transplanted line. International protocols governing the ethical treatment of animals were followed. Mice were divided into six groups and each group contained ten animals. Group I (G I) served as negative control animals received saline solution intraperitoneal (i.p.) (0.9%), and was fed standard pellet diet. Group II (G II) was transplanted to the i.p. cavity with EACC at $1x10^6$ cells (0.2 ml) (positive control). Group III (G III) was transplanted with EACC like the second group and each mouse was daily forced to ingest orally *via* stomach tube about 0.2 ml of aloesin (10% w/v) in addition to the normal diet. Group IV (G IV) was treated daily like the third group but with barbaloin. Group V (G V) was treated daily like the third group but with octapeptide (10% w/v). Group VI (G VI) was treated daily like the G III but with aloe-emodin. Total experimental period was 40 days after mice were transplanted with EACC *via* the IP cavity (adaptation period). Body weight was recorded weekly and life span was calculated after the experiment period. The number of EACC cells was calculated weekly in all animals. Cells of EACC were obtained from transplanted animals and then washed with three changes of Phosphate Buffer Saline (PBS). The cells were tested for their viability using the trypan blue exclusion test [27].

2.11. Biochemical Analysis

Antioxidant enzyme activity was determined in negative control EACC-transplanted positive control group and EACC-administered groups treated with different *A. vera* active principals. At the end of the experimental period, animals were sacrificed by cervical decapitation and blood was collected. Enzyme activities of superoxide dismutase (SOD), total glutathione peroxidase (tGPx) and glutathione S-transferase (GST) were assayed as previously described [30-32]. Lactic dehydrogenase (LDH) activity was determined weekly in EACC which were separated from mice [33].

3. RESULTS AND DISCUSSION

3.1. Effect of *A. vera* Active Principles on Animal Life Span and Body Weight

Ehrlich ascite carcinoma (EACC) tumor in mice has previously been employed as a model for rapidly growing tumors, where various experimental agents can be applied and tested for biological activity as potential anticancer and antitumor substances [26,34]. A detailed monitoring of body weight and life span/survival of animals before and after tumor transplantation was carried out. In the positive control groups (G II), which was administered with $1x10^6$ EACC cells per animal, there was a premature death by the second week at 14 days post-transplantation, and this correlated with a significant increase in the net body weight of EACC-administered animals by 50% (3 g) compared to saline-administered animals (6 g in GII and 3 g in G I). This can be explained by the uncontrolled growth of the tumor cells and the increase in their biomass (Fig. **2A**). Strikingly, there was a significant inhibition on the growth of EACC tumor in mice, as judged by the net body weight, which correlated well with *A. vera* active principles treatment (Fig. **2A**). Active principles that gave the strongest inhibitory effect on EACC tumor growth, as judged by average animals body weight compared to saline-administered group (25 g), by the third week, were in the following order: barbaloin (G IV, 25 g, i.e. -1 g) > octapeptide (G V, 29 g, i.e. +4 g) > aloesin (G III, 30 g, i.e. +5 g) > aloe-emodin (G VI, 31 g, i.e. +6 g) (Fig. **2A**). Interestingly, these results were accompanied with delayed death of animals and prolongation of the survival period in days (Fig. **2B**). In the positive control group, which was only injected with EACC cells, an intensive mice death was detected after 14^{th} day-post-injection (dpi) because of the tumor (Fig. **2B**). Active principles with the strongest impact on the prolongation of the life span tumor-transplanted animals were in the following order: barbaloin (G IV, 36 dpi) > octapeptide (G V, 28 dpi) > aloesin (G III, 25 dpi) > aloe-emodin (G VI, 21 dpi) (Fig. **2B**). Hence, it can be concluded that all four active principles exerted a significant *in vivo* inhibitory effect on the growth of EACC tumor progression, which correlated well with the prolongation of animals' life span. These results are in agreement with previously reported

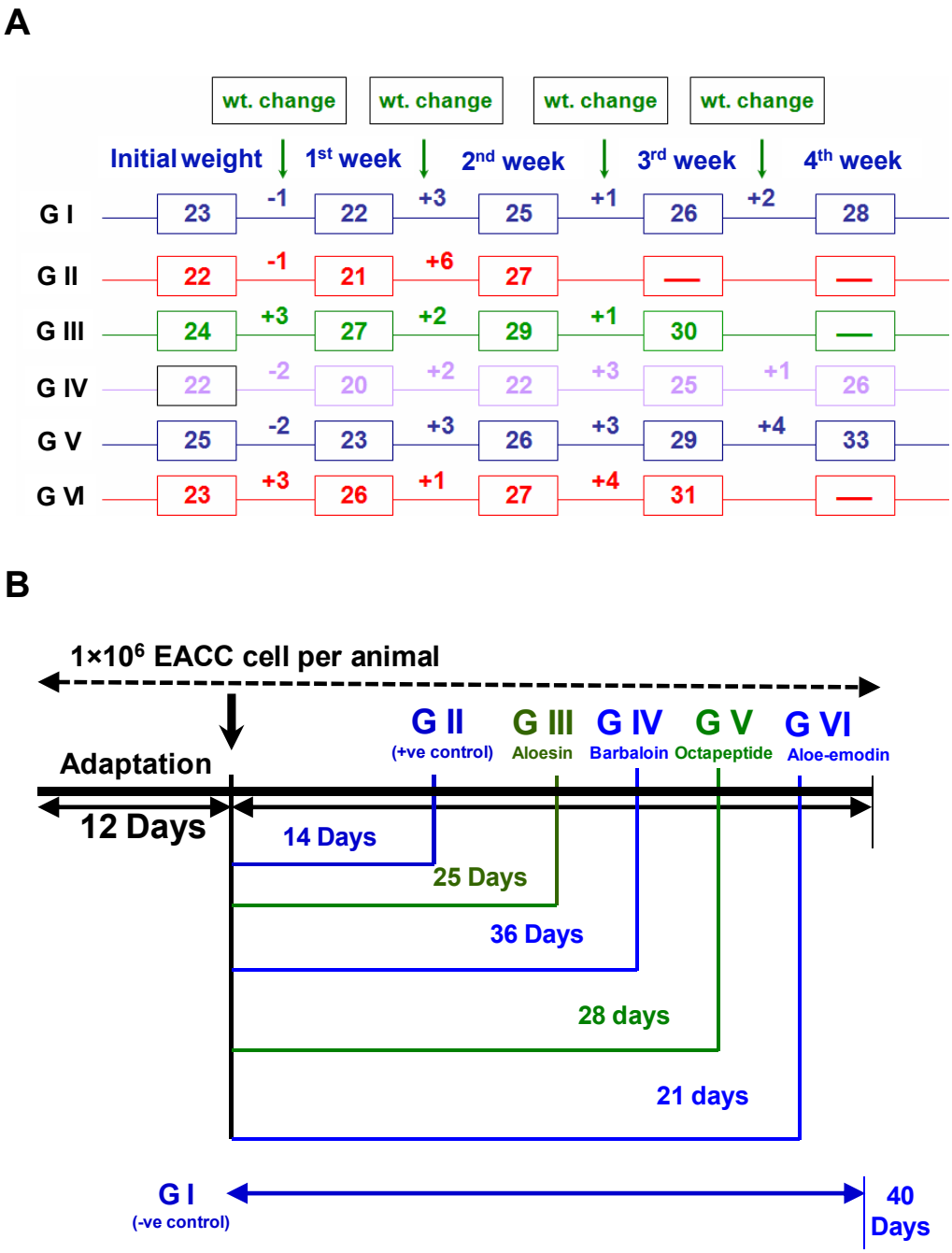

Fig. (2). Effect of different active principles of *A. vera* leaves on body weight (**A**) and life span (**B**) of tumor-transplanted animals. Animals were divided into 6 groups and administered the respective treatment, as detailed in Materials and Methods section. Total experimental period was 40 days after mice were transplanted with EACC ($1x10^6$ cells per animal) *via* the intraperitoneal cavity (adaptation period).

studies of the effect of natural phytochemical extracts and/or active principles as efficient antitumor agent [26,34].

3.3. *In Vitro* Effects of *A. vera* Active Principles on Human Tumor Cell Lines

In anticancer assay, two kinds of typical human cancer cell lines were selected to evaluate the anticancer activity of the extracted compounds on growing cancer cell lines. Cancer cell lines that were employed include: two human leukemia cancer cell types (AML and ALL) and one type of animal tumor cells (EACC). The four purified active principles from *A. vera* leaf extracts (Namely: barbaloin, octapeptide, aloesin and aloe-emodin) were tested for their antileukemic activity on AML and ALL cells. After removing the solvent,

active principles were dissolved in a saline solution, incubated with the leukemia cells and their *in vitro* inhibitory potential on the growth of human tumor cells was studied using trypan blue exclusion test [27]. Also we tested the isolated compounds on normal bone marrow cells to evaluate the specificity of the cytotoxic effect of the isolated compounds. It was concluded that the active principles at the concentrations used are non-toxic to normal bone marrow cells (3.2%- 3.7%) when compared to 3.1% in the control (negative control).

The results summarized in Table **1** and Table **2** clearly indicate that tested active principles exhibited a significant dose-dependent inhibitory effect on all cancer cell lines examined, with varying effect on the different cell lines. Results indicated that in human AML and ALL cells, treated with these natural compounds at increasing concentrations (50-200 μg ml^{-1}) for 24 h, aloe-emodin and aloesin exhibited the strongest cytotoxic activities, followed by octapeptide and barbaloin, respectively (Table **1**). For example, approximately a 50% increase in the cytotoxicity, against both AML and ALL cells, was observed when the concentration of aloe-emodin and aloesin was increased from 50 to 200 mg ml^{-1} (Table **1**). At a concentration of 200 μg ml^{-1}, aloe-emodin showed a cytotoxicity of 95±4.25% and 94±1.61% against AML and ALL cells, respectively, while aloesin exhibited cytotoxic values of 90±1.35% and 91±2.94% against AML and ALL cells, respectively (Table **1**). Similarly, the viability of EACC tumor cells, after incubation with isolated compounds were significantly affected with cytotoxic values at the maximum concentration of 200 μg ml^{-1} for the four compounds in the following order: aloesin (84±4.12%), octapeptide (80±3.56%), barbaloin (77±2.45%) and aloe-emodin (59±3.2) (Table **2**). Moreover, the effect of active principles on EACC tumor cells number in two-week-post-injection was investigated (Table **3**). *A. vera* active principles exhibited significant inhibition on EACC tumor cell number, when compared to positive control group (479±6.52), in the following order: barbaloin (32±1.57)> aloe-emodin (59±3.45)> octapeptide (80±2.52)> aloesin (105±4.52) (Table **3**). Taken together, it is clear that the observed significant antitumor activity, particularly for aloe-emodin and barbaloin, is promising against the three types of tumor-cell-types studied.

We further verified the anticancer activity of the SFE-extracted aloe-emodin and barbaloin, against two more human cancer colon cells (DLD-1 and HT-29), by MTT assay. Aloe-emodin was found to be active against both DLD-1 and HT29 cell lines, with IC_{50} values of 8.94 and 10.78 μM, respectively (Fig. **3A** and **B**), while barbaloin exhibited no activity (IC_{50} > 100 μM), which can be attributed to its hydrophilic nature. The IC_{50} of 5-Fluorouracil (positive control) against DLD-1 was 7.38 μM.

Table 1. ***In Vitro*** **Effect of Isolated** ***A. vera*** **Active Principles on the Cytoviability (%) of Human AML and ALL Cells After 24 h Incubation Period**

Compound	Extract concentration (μg ml^{-1})															
	AML								ALL							
	50		100		150		200		50		100		150		200	
	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead
Octapeptide	46±1.10	54±1.90	31±2.38	69±1.23	18±2.13	82±2.58	15±3.67	87±3.70	26±1.35	74±1.81	22±2.16	78±2.33	17±2.10	83±2.05	14±1.15	86±1.67
Aloesin	39±1.80	61±1.60	19±1.60	73±2.1	18±2.66	82±1.05	10±1.36	90±1.35	25±1.85	75±2.86	20±1.67	80±2.3	15±1.95	85±1.69	9±1.66	91±2.94
Barbaloin	54±1.85	36±1.15	41±1.25	59±2.31	28±1.90	72±1.78	19±3.57	81±2.15	43±2.50	57±2.54	29±1.92	71±2.7	23±2.21	77±2.57	18±1.58	82±2.71
Aloe-emodin	43±1.48	57±1.58	14±2.10	86±3.56	8±0.45	92±3.21	5±2.32	95±4.25	24±2.51	76±1.20	20±1.77	80±1.76	11±1.72	89±1.21	6±1.04	94±1.61

-EACC value represents the mean of three replicates.

Table 2. ***In Vitro*** **Effect of** ***A. vera*** **Active Principles on the Cytoviability (%) of Mice EAC Cells After 24 h Incubation Period**

Compound	Extract concentration (μg ml^{-1})							
	EACC							
	50 μg		100 μg		150 μg		200 μg	
	Viable	Dead	Viable	Dead	Viable	Dead	Viable	Dead
Octapeptide	71±2.13	29±2.50	66±2.30	34±2.36	55±2.13	45±1.35	20±2.36	80±3.56
Aloesin	73±3.12	27±1.32	45±1.56	55±3.25	33±1.45	67±4.21	16±.23	84±4.12
Barbatoin	60±1.26	40±2.43	49±2.35	51±1.89	42±2.45	58±2.52	23±2.40	77±2.45
Aloe-emodin	69±1.67	31± 1.25	57±3.25	43±2.65	46±2.58	54±2.64	41±3.12	59±3.2

-EACC value represents the mean ± SD (Standard Deviation) of three replicates.

Table 3. ***In Vivo*** **Effect of Isolated** ***A. vera*** **Active Principles on EAC Tumor Cell Number in Mice**

2nd week (×10^6)	1st week (×10^6)	Group (n)
479±6.52	83±3.58	Positive control (G II)
105±4.52*	84±1.25	Group (GIII)
32±1.57*	79±3.56	Group (GIV)
80±2.52*	82±2.50	Group (GV)
59±3.45*	86±3.89	Group (GVI)

-EACC value represents the mean ± S.D (Standard Deviation) of three replicates.
-*Values are significantly different from the positive control at ($P \leq 0.05$).

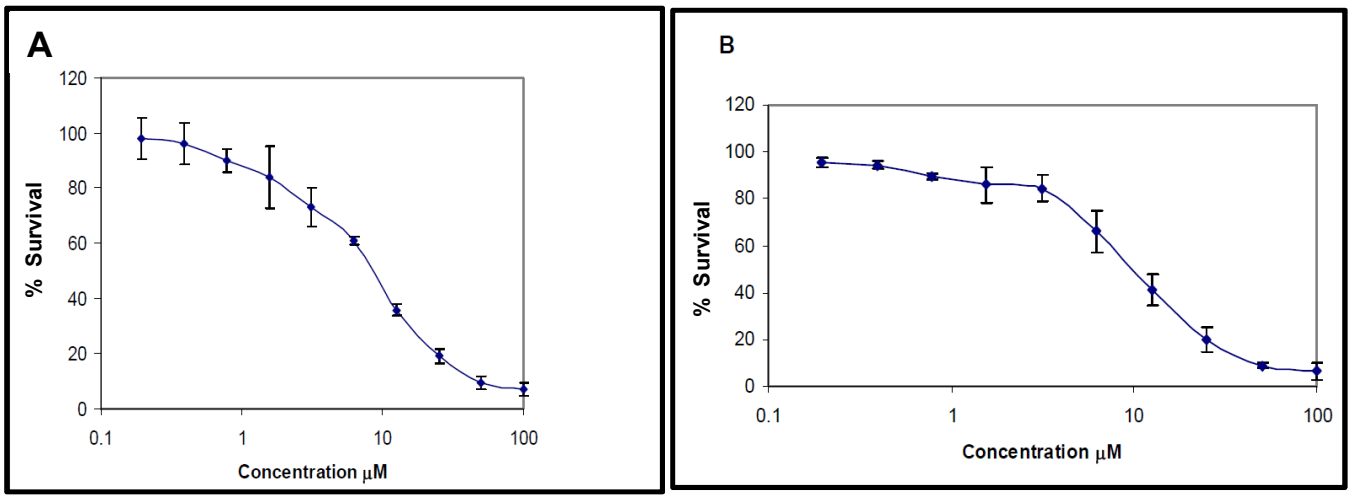

Fig. (3). Cell viability dose-response curve of aloe-emodin against DLD-1 cells (**A**) and HT-29 cells (**B**), according to MTT assay. Results are expressed as mean % cell survival ± standard deviation of three independent experiments.

The results presented here showed the cytotoxic activity of alo-emodin against five different types of cancer cell lines. Our data are in agreement with previous reports stating that aloe-emodin possesses a versatile anticancer activity against various tumor cell types including: neuroectodermal tumor cells, neuroblastoma cells [35], human lung carcinoma cells [36], merkel carcinoma cells [37] and hepatoma cells [38]. Moreover, we report, for the first time, the potent anticancer effect of a novel group of *A. vera*-derived natural active principles such as aloesin, octapeptide and barbaloin. Moreover, our data also suggest that for each anticancer drug there is a cell-type specificity range of activity that should be taken into account prior to implementation in cancer therapy programmes. This is indicated by the fact that barabloin exhibited a strong and significant cytotoxicity against leukemic animal (EACC) and human (AML and ALL) cancer cells but was not active against human cancer colon cells ((DLD-1 and HT-29).

3.4. Effect of *A. vera* Active Principles on Antioxidant Enzymes

All cells possess elaborate antioxidant defense systems that consist of interacting low and high molecular weight components. Among them, superoxide dismutases (SOD), total glutathione peroxidases (tGPx), and Glutathione S-Transferase (GST) play a central role. Antioxidant enzymes can antagonize initiation and promotion phases of carcinogenesis and they are reduced in many malignancies.

Significantly elevated lactate dehydrogenase (LDH) activity level (($P \leq 0.05$) was observed when EACC tumor-transplanted mice was treated with *A. vera* active principles in the following order: barbaloin (33.17±2.03)> aloe-emodin (23.49±1.02)> aloesin (20.23±1.26), but not with octapeptide (14.84±1.09), as compared to positive control group (12.14± 1.85) (Table **4**). The prognostic role of LDH has been widely investigated in special cancer groups. Elevated LDH is consistently reported as a prognostic factor for poor survival in various cancers [39; and references therein]. Thus, The release of the intracellular LDH, which mediates the conversion of 2,4-dinitrophenylhydrazine into a visible hydrazone precipitate in the presence of pyruvate, have previously been measured and used as a biomarker of membrane integrity loss in dying cells [40].

Significantly enhanced GST activity level ($P \leq 0.05$) was observed with *A. vera* active principles in the following order: barbaloin (30.30±1.06)> aloe-emodin (29.05±0.06) but not with aloesin or octapeptide, as compared to positive control group (20.92± 0.76) (Table **4**). GST is an enzyme involved in antioxidant defence and also involved in detoxification. It is used as a tumor marker in certain cancers such as oral cancer. Alterations in GST levels in tumor tissue have been reported by various studies [41, 42].

Table 4. Biochemical Impact of Isolated *A. vera* Active Principles Antioxidant Enzymes (GST, tGPx and SOD) and LDH Levels in Serum of EAC Tumor-Transplanted Experimental Animals as Compared to Control Group

SOD activity (U ml^{-1})	tGPx activity (mU ml^{-1})	GST activity U L^{-1}	LDH activity (U L^{-1})	Group (n)
82.8 ± 1.84	95.14±0.01	53.11± 0.01	22.9±0.04	Negative control (G I)
28.12± 2.08	56.13±1.85	20.92±0.76	12.14±1.85	Positive control (G II)
70.31±0.23*	67.43±0.03*	15.62±0.36	20.23±1.26*	Group (G III)
85.93±1.06*	92.94±1.09*	30.30±1.06*	±2.03*33.17	Group (G IV)
32.06±0.32	60.52±0.98	19.37±0.96	14.84±1.09	Group (G V)
82.81±2.09*	64.84±0.12*	29.05±0.06*	23.49±1.02*	Group (G VI)

-EACC value represents the mean ± S.D (Standard Deviation) of three replicates.
*Values are significantly different from the positive control at ($P \leq 0.05$).

We further evaluated the influence of *A. vera* active principles on the level of serum SOD activity in EACC tumor cells. SOD being the first lines of defence against ROS-mediated oxidative damage, catalyses the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) that can be transformed into H_2O and O_2 by catalase. Hence, we found a significant elevation ($P \leq 0.05$) in serum SOD activity in response to the *A. vera* isolated compounds in the following order: barbaloin (85.93±.1.06)> aloe-emoedin (82.81±2.09)> aloesin (70.31±0.23), as compared to positive control (28.12±2.08) (Table **4**). It has been reported that SOD activity is reduced in a variety of tumor cells and has been proposed to be a new type of tumor suppressor gene [43,44].

Similarly, tGPx activity was found to be significantly increased ($P \leq 0.05$) in response to *A. vera* active principles in the following order: Barbaloin (92.94±.1.09)> aloesin (67.43±0.03)> aloe-emoedin (64.84±0.12), as compared to positive control (56.13±1.85) (Table **4**). tGPx is a selenoprotein, which reduces lipidic or nonlipidic hydroperoxides as well as H_2O_2 while oxidizing glutathione. In this context, increased GSH-Px and glutathione activities have been reported in patients with leukemias [45,46].

Taken together, the significant elevation observed in key antioxidant enzyme activities, in response to *A. vera* active principles, could be related to a therapeutic modulatory effect in an attempt to rectify an unbalanced alteration in oxidant–antioxidant status. Thus, it has been documented that the antioxidant system is impaired as a consequence of an abnormality in the antioxidative metabolism due to the cancer process [47].

3.5. Effect of *A. vera* Active Principles on DNA Fragmentation

Obtained results showed a potent antiprofilerative and cytotoxic effects of *A. vera* isolated active principles on cancer cells. To verify whether the observed potent anitprofilerative is mediated through apoptosis, *in situ* DNA fragmentation analysis was performed (Fig. **4**). Treatments of human AML leukemic cells with active principles (100 μg ml^{-1}) resulted in a varying intensities of internucleosomal DNA fragmentation, evidenced by the formation of a DNA ladder on agarose gel, hallmark of cells undergoing apoptosis, in the following order: aloe-emodin> aloesin> barbaloin> octapeptide (Fig. **4**). No DNA laddering was detected in the sample from control cells. Our results conform to previous findings indicating that aloe-emodin results in an apoptotic cell death [38,48]. Moreover, it has been reported that the resulting DNA damage is mediated through through generation of ROS in human lung carcinoma cells [48].

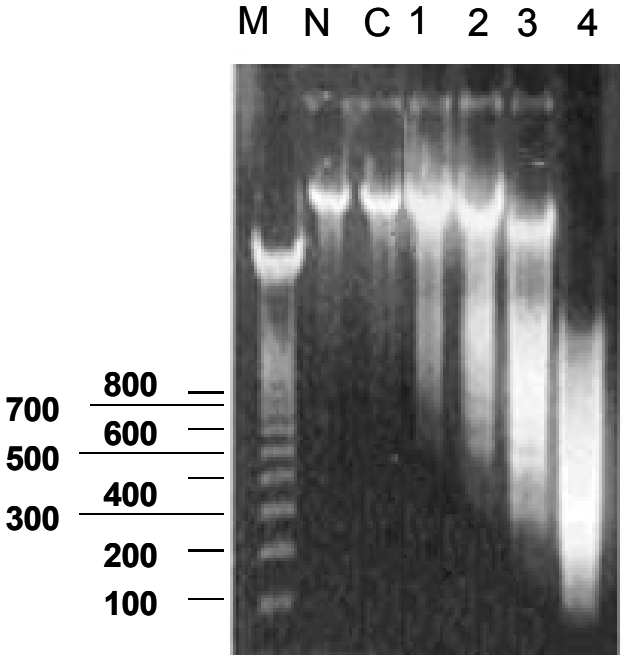

Fig. (4). Induced internucleosomal DNA fragmentation in AML cells treated with isolated *A. vera* active principles. Cells were incubated with the compounds in 0.1% DMSO for 24 h in 1% serum-containing medium. After treatment, cells were lysed and total cellular DNA was extracted and electrophoresed on a 2% agarose gel containing 0.01 mg ml^{-1} ethidium bromide at 5 V/cm. M: represents molecular DNA maker expressed in base pairs; N: normal bone marrow cells; C: untreated AML cells; Lane 1: AML cells treated with octapeptide (100 μg ml^{-1}); Lane 2: AML cells treated with barbaloin (100 μg ml^{-1}); Lane 3: AML cells treated with aloesin (100 μg ml^{-1}); Lane 4: AML cells treated with aloe-emodin (100 μg ml^{-1}). Results are representative of three independent experiments.

In conclusion, the anticancer activity, which is based on apoptotic cell death, is promoted by a tumor cell-specific drug uptake process (e.g. aloe-emodin and barbaloin) that

may offer opportunities for novel anticancer agents. Importantly we demonstrated that the tested *A. vera* active principles exhibit selectivity towards cancer profilerating cells as well as a non-toxic behavior towards normal hematopoietic progenitor cells. This key advantage is lacking in common anticancer drugs exhibiting a non-selective mechanisms of action, with an inevitably high incidence of severe toxicity must be tolerated for effective doses to be administered [49]. We envisage that this finding, among others, should point to novel low-cost drug discoveries, of natural origin, which can be afforded by developing countries. More efforts should be focused especially on the clarification of the mechanisms of action of the components of herbs, which is vital for developing their potential applications in treating terminal diseases like cancer.

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