Comparative Study on the Anticancer Drug Potential of a Lectin Purified from Aloe Vera and Aloe-Emodin

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Abstract

Background: The effect of Aloctin, a lectin purified from Aloe vera leaves, and aloe emodin an anthraquinone glycoside purified from the leaves of the same plant, on several cancer cell lines was investigated. Methods: Aloctin was isolated from A. vera leaf skin by ammonium sulphate precipitation and CNBr-Sepharose 4B-ovalbumin affinity chromatography. Specific new ligands for Aloctin were detected as fetuin and avidin by hemagglutination inhibition tests. The cytotoxic effect of Aloctin and aloe emodin on various human cancer cell lines was tested using MTT assay. Imatinib was tested as standard positive control. The mechanism underlying was tested by the Annexin V-FITC/PI test, with flow cytometry. Results: The most sensitive cells to Aloctin and aloe emodin treatment, were identified as AGS human gastric adenocarcinoma cells. The effect was concentration dependent. It was shown that this effect does not occur by apoptosis or necrosis. In Aloctin-imatinib combinations studies, Aloctin significantly increased the cytotoxic effect of imatinib in a dose-dependent manner. It is expected that the results of this study will reveal important findings for the future use of A. vera lectin as well as aloe emodin in cancer research and contribution to lectin biochemistry.

Keywords: Aloe- emodin- cytotoxicity- gastric adenocarcinoma- lectin

Introduction

As cancer continues to be one of the devastating illnesses of our century, plant kingdom with its diversity, is also researched in regard of cytotoxic and anticancer agents. Chemotherapeutic drugs of plant origin are promising strategy for cancer therapy because they might be generally harmless or less toxic than synthetic chemotherapeutic agents on normal cells.

Lectins are proteins or glycoproteins that bind to specific sugar residues on cell surfaces (Sharon and Lis, 1972). Lectins play multiple roles in inter- and intra-cellular signaling, cell transformation and cell adhesion (Seyrek and Bildik, 2001). It is reported that cell surface carbohydrates take place in normal and malignant transformations. Changes in cell surface glycoproteins were reported during malignant transformation (Diani, 2010). Bovin and Gabius (1995) proposed that tumours could be better recognized by some glycoprotein-binding lectin markers. Lectin-targeted chemotherapy as well as lectin binding studies for normal and tumour cells are thus promising strategies for the future (Gorelik et al., 2001). Nevertheless direct antitumour and cytotoxic effect of some lectins is also reported in literature (Hajtó et al. 2005; Akev et al., 2007b; Faheina-Martins et al 2012).

The present study was undertaken in order to determine and to compare the cytotoxic effects of Aloctin, purified by CNBr-Sepharose 4B-ovalbumin affinity chromatography, and AE on several cancer cell lines.

Materials and Methods

Plant Material

Specimens of A. vera (L.) Burm. f. were collected from Kale (Demre) in Antalya (May 1993) and cultivated in the Greenhouse of Istanbul University Alfred Heilbronn Botanical Garden. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, ISTE No. 65118. The fresh leaves of this cultivated plant were used

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Preparation of *A. vera* leaf extract

Freshly chopped *A. vera* leaves, were washed carefully with water and dried with filter paper (Whatmann 41) to remove dust and foreign materials. The leaves were put vertically in a becher for one night in order to discard the brown latex rich in anthraquinones. Then the leaves were longitudinally split in two, the gel was separated by scraping with a spoon, the leaf skins were cut into little pieces and homogenized with phosphate buffered saline (PBS) in a Waring blender. The homogenate was filtered through cloth and then filtrate was centrifuged at -10°C, 10,000 rpm for 30 min. The clear supernatant was lyophilized and considered to be “*A. vera* leaf skin crude extract”.

Purification of *A. vera* lectin

The lectin was precipitated by adding 50% ammonium sulphate to the *A. vera* leaf skin crude extract solution. The precipitate was dissolved in a minimum amount of PBS, dialyzed against PBS and centrifuged. The clear supernatant was named “50% ammonium sulphate cut”.

Affinity chromatography on CNBR-activated Sepharose 4B-ovalbumin

50% ammonium sulphate cut was applied to affinity chromatography on a column of CNBR-activated Sepharose 4B-ovalbumin. The protein peak of the eluate showing hemagglutination was collected, concentrated, lyophilized and the purity was assessed by polyacrylamide gel electrophoresis as described previously (Ozsoy et al., 2012).

Hemagglutination inhibition tests

They are hapten inhibition tests performed in order to investigate the inhibition of lectin-induced hemagglutination and thus find the proper ligand of the lectin. The hemagglutination inhibition tests by various carbohydrates were performed in a manner analogous to the hemagglutination test (Wang et al. 1995; Akev and Can 1999). The carbohydrates used were: D(+)-raffinose, mellibiose, D-mannose, N-acetyl-D-galactosamine and chitin. The glycoproteins used were: fetuin, avidin, musin and inulin.

Preparation of test materials and reference drugs

Aloe emodin (AE) (1,8-dihydroxy-3-[hydroxymethyl]-anthraquinone) was purchased from Sigma (St Louis, MO, cat no. A7687). AE (20 mmol/L) stock solutions were prepared in DMSO, aliquoted and stored in the dark at -20°C till use, diluted with medium. Imatinib (IM) was a kind gift from Istanbul University, Istanbul Faculty of Medicine, Department of Physiology.

Cell lines and cell culture

AGS human gastric adenocarcinoma, HCT116 human colon cancer, HEP3B human hepatoma, HL60 human acute promyelocytic leukemia, K562 human chronic myelogenous leukemia and Saos-2 human osteosarcoma cell lines were a courtesy of Prof. Dr. Serap Erdem Kuruca, Istanbul University, Istanbul Faculty of Medicine, Department of Physiology. The cells were cultured in DMEM (Dulbecco’s Modified Eagle’s medium; Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Capricorn FBS-12A), 100 units/mL penicillin and 100 μg/mL of streptomycin in a humidified incubator containing 5% CO₂ at 37°C. In order to reach the sufficient cell number for tests, cells were passaged after reaching 80% monolayer confluency. Cells were sub-cultured every 2 or 3 days.

Trypan Blue exclusion assay

The total number of viable cells was determined at each time point by the trypan blue exclusion test (Strober 2001). Exactly 10 μL of cell suspensions was stained with an equal volume of trypan blue (0.4% in 10 mM phosphate buffer saline) for 1 min. Then the numbers of viable cells were counted with Neubauer Chamber by light microscopy. Cells that retained a blue color were considered as dead cells.

MTT colorimetric assay

The MTT colorimetric assay developed by Mosmann 1983 with modification was used to screen for cytotoxic activity. For this purpose 96-well plate was used and the assay was done in a total volume of 100 μL. Briefly, 10 μL/well of varying concentrations of AVG and AE, (50 - 250 μg/mL; 10 - 80 μM respectively) were added and subsequently the cells (90 μL/well; 105 cells/mL culture medium) were seeded to treate for 72 h. After the aspiration of supernatant (50 μL/well), incubation with MTT solution (10 μL of 5 mg/mL PBS) at 37°C for 3 h, cells were lysed with 100 μL dimethyl sulfoxide (DMSO). The yellow MTT dye was reduced by succinic dehydrogenase in the mitochondria of viable cells to purple formazan crystals. Absorbance was measured at 570 nm using a microplate reader.

To account for absorbance of samples at 570 nm, during each MTT experiment, separate wells were set where samples were diluted in culture medium without cells. The average absorbance readings from wells containing samples in culture medium were subtracted from the readings of treated cells. To calculate viability index, absorbance readings from DMSO treated control wells were set at 100% and the relative absorbance was calculated as a percentage of the control.

The results were generated from three independent experiments; all experiments were performed in triplicate. Cytotoxic index was expressed as a percentage relative to the untreated control cells.

The cytotoxic concentrations of extracts that provides 50% inhibition of cell growth (IC₅₀) were calculated from dose-response curve. The cytotoxic effect of *A. vera* extracts and controls were evaluated by comparing the IC₅₀ Values of cell lines.

Flow cytometry analysis

Fluorescein (FITC) Annexin V-/Propidium iodide (PI) double labeling was performed with the Annexin V-FITC apoptosis detection kit (Millipore) to detect the apoptosis of cells. For this purpose 6-well plate was used and the
assay was done in a total volume of 2 mL. The three groups (two untreated control cell group: to apply and unapplied Annexin V-FITC/PI for one test group) of cells (1800 µL/well; 10^5 cells/mL culture medium) were seeded in 6-well plates in a final concentration of IC_{50} (200 µL/well) of A. vera gel extract and AE. After culture at 37°C with 5% CO_2 for 72 h, the cells were harvested by trypsinization. Prior to trypsinization, floating or loose cells were harvested by gentle manual rocking of the culture dishes and transferring the culture medium containing the cells into centrifuge tubes. Trypsinized and loose cells were then combined and pelleted by centrifuging at 2000 rpm for 10 min. The pellets were resuspended and washed with PBS, then resuspended in 100 µL of Annexin Binding Buffer (4X) and stained with 3 µL Annexin V-FITC, 2 µL PI. The cell suspension was incubated for 45 min at room temperature in the dark. The cell suspension was then immediately analyzed by flow cytometry. Cell Quest software was used to analyze 10^4 cells. The apoptotic cells were determined with a FACS Calibur flow cytometer (BD Biosciences) and analyzed with CELLQUEST software (BD Biosciences).

### Statistical analysis

The results were statistically analyzed using the independent Student’s t-test. Data were represented as means ± standard deviation (S.D.) and at least in triplicate. Results were considered significant with P < 0.05 (**), P < 0.01 (**) ve P < 0.001 (***)

### Results

**Affinity chromatography purification of A. vera lectin (Aloctin)**

The affinity chromatography elution profile of Aloctin purification is shown in Figure 1. The lectin was eluted as a single peak. The results of Aloctin purification steps are shown in Table 1.

![Figure 1. Elution Profile of Aloe vera Lectin (Aloctin) Purification. Affinity chromatography of 50% ammonium sulphate precipitate of Aloe vera leaf skin extract using CNBr- Sepharose 4B- ovalbumin. HA, Hemagglutination activity; Column size, 25x0.5 cm; Flow rate, 4 ml/min; ---Absorbance (280 nm); HA, Fractions showing hemagglutination activity.](image1)

![Figure 2. Cell Viability (%) Level Measured by MTT Assay after 72 Hours Treatment with Different Concentration of Aloctin (A) and Aloe Emodin (B) on Cancer Cells. 400, 800 and 1000 µg/ml concentrations showed significant differences, compared to control group. AGS human gastric adenocarcinoma cells, HCT116 human colon cancer cells, HEP3B human hepatoma cells, HL60 human acute promyelocytic leukemia cells, K562 human chronic myelogenous leukemia cells, Saos-2 human osteosarcoma cells. *P < 0.05; **P < 0.01; ***P < 0.001.](image2)

**Table 1. Purification Steps of Aloctin from A. vera Leaf Skin Extract (Starting from 103 g A. vera leaf skin)**

| Purification step          | Volume (ml) | Protein (µg/µL) | Total protein (µg) | HA titer | HU/mg | Purification |
|----------------------------|-------------|-----------------|--------------------|----------|-------|--------------|
| Crude extract              | 200         | 41.30 ± 0.73    | 8260.3 ± 46.3      | 8        | 12.8  | 1            |
| 50 % ammonium sulphate cut | 25          | 162.87 ± 3.19   | 4071.86 ± 79.66    | 8        | 51.2  | 4            |
| Affinity chromatography    | 7.9         | 151.69 ± 4.38   | 1198.31 ± 34.10    | 32       | 192.8 | 15.06        |

HA, hemagglutination; HU, hemagglutination units
None of the carbohydrates tested showed inhibition of hemagglutination. Only N-acetyl D-galactosamine showed weak inhibition as found previously (Akev and Can 1999). Among the glycoproteins tested fetuin and avidin significantly inhibited hemagglutination activity of Aloctin and their minimum inhibitory concentrations are 0.156 mg/ml and 0.078 mg/ml, thus are potential ligands for further studies with Aloctin. Results of hemagglutination inhibition test are shown in Table 2.

### Cytotoxicity of Aloctin against cancer cell lines

The cytotoxic concentrations of Aloctin that provides 50% inhibition of cell growth (IC$_{50}$) are shown in Table 3. The best effect of IM was seen on K562 cells whereas the effect of AE could be ranged as AGS > HL60 > Saos-2 > K562 > HCT116 > HEP3B cells.

### Cytotoxicity of aloe emodin against cancer cell lines

The cytotoxic effect of AE on the cells as percentage of viable cells, is shown in Figure 2B. Significant cytotoxic effect was shown with treatment of AGS cells with AE after 10 µM concentration (P < 0.01). On
HCT116 cells significant cytotoxicity was observed only at higher doses of 60-150 µM (P < 0.01). On HEP3B cells significant cytotoxicity was observed after doses of 20 µM (P < 0.01). Cytotoxic activity of AE on HL60 cells was significant between 20-80 µM (P < 0.01). AE showed significant cytotoxicity on K562 cells at 20 µM (P < 0.01) and between 40 – 80 µM (P < 0.001) concentrations. AE showed significant cytotoxicity towards Saos-2 cells at lower doses of 10 µM (P < 0.05), and also at higher doses of 20 µM (P < 0.01), 40 µM (P < 0.001), 60µM (P < 0.001) and 80 µM (P < 0.01).

Cytotoxicity of Alocitin-Imatinib combination against cancer cell lines

Alocitin-IM combinations were assayed on AGS human gastric adenocarcinoma and Saos-2 human osteosarcoma which were the two most sensitive cells to Alocitin. The results of the Alocitin-IM combination treatment of AGS and Saos-2 cells are presented in Table 4.

For AGS cells 1 µg/mL Alocitin combined with 50 µM IM, exerted significant difference (P < 0.05) in cytotoxic activity compared to the same dose of IM alone (Figure 4A). The results were more spectacular for Saos-2 cells, for which significant enhance in cytotoxicity compared to the same dose of IM alone was found for 0.5 µg/mL Alocitin combined with 25 µM IM (P < 0.001) as well as 1 µg/mL Alocitin combined with 50 µM IM (P < 0.01) (Figure 4B).

Apoptosis/Necrosis analyses undertaken by flow cytometry

The results of Annexin V-FITC/PI assay apoptosis/necrosis analyses of the activity of Alocitin on cancer cells is are summarized in Figure 5. The results of flow cytometric studies on Alocitin treated cells revealed that

Table 3. IC₅₀ Values of Alocitin, AE and IM on Cells

| Cell line | Alocitin (µg/mL) | AE (µM) | IM (µM) |
|-----------|-----------------|---------|---------|
| AGS       | < 0.3           | 19.03 ± 0.25 | 65 ± 1.31 |
| HCT116    | 1.42 ± 0.07     | > 150    | 50 ± 2.60 |
| HEP3B     | 0.55 ± 0.02     | 201.64 ± 1.43 | 75 – 100  |
| HL60      | 1.03 ± 0.07     | 20.93 ± 1.96 | 25 ± 0.51 |
| K562      | 0.97 ± 0.06     | 60.98 ± 0.90 | 10 ± 0.34 |
| Saos-2    | <0.3            | 33.44 ± 0.68 | 108 ± 5.62 |

Table 4. Results of IM-Alocitin Combination Tests for AGS and Saos-2 Cells

| Treatment dose | Viable cells (%) |
|----------------|------------------|
|                | AGS   | Saos-2 |
| 25 µM IM       | 57.37* | 68.72* |
| 50 µM IM       | 47.28* | 50.90* |
| 0.5 µg/mL Alocitin | 33.10** | 30.89* |
| 1 µg/mL Alocitin | 18.65** | 33.54 |
| 25 µM IM+0.5 µg/mL Alocitin | 15.74** | 33.11* |
| 50 µM IM+1 µg/mL Alocitin | 9.66*** | 15.20* |

The tests were done in triplicate Student’s t test was applied in comparison to control untreated cells. *P < 0.05; **P < 0.01; ***P < 0.001.

Figure 4. Cytotoxic Effect of Alocitin-IM Combination Treatment on A) AGS Human Gastric Adenocarcinoma Cells. B) Saos-2 human osteosarcoma cells. Cell viability (%) level measured by MTT assay after 72 hours treatment with different concentration of Imatinib (IM; 25 µM, 50 µM), Alocitin (0.5 µg/mL, 1 µg/mL) and combination of IM and Alocitin (25 µM + 0.5 µg/mL, 50 µM IM + 1 µg/mL). 25 µM IM + 0.5 µg/mL Alocitin concentration showed significant differences, compared to 25 µg/mL IM group in AGS cells. 0.5 µM IM + 1 µg/mL Alocitin concentration showed significant differences, compared to 50 µg/mL IM group in AGS and Saos-2 cells. *P < 0.05; **P < 0.01; ***P < 0.001.
Aloctin did not exert its cytotoxic activity by apoptosis or necrosis mechanisms.

Discussion

As every lectin binds to specific carbohydrate residues or sugar moieties of glycoproteins situated on cell surfaces,
it is important for research in lectin histochemistry, as well as for further lectin purification by affinity chromatography, to determine the specific ligand of every lectin. In our previous researches only N-acetyl galactosamine (Akev and Can, 1999) and ovalbumin (Ozsoy et al., 2012) were found to inhibit hemagglutination of Aloctin. Ovalbumin was thus used in affinity chromatography purification of Aloctin. In the present study, in a search for a new ligand, none of the different simple carbohydrates assayed has shown inhibition of hemagglutination. Among the various glycoproteins used, the fact that fetuin, avidin and musin have shown hemagglutination inhibition, tend us to propose these substances in further research as ligands for Aloctin.

In previous years lectins were presented as toxic substances, but nowadays this nocive property could be a chance for their use as antitumour agents. It was shown that lectins play role in apoptosis and autophagy (Liu et al., 2010.). As new strategies, especially based on plant derived chemotherapeutic agents have begun to emerge, research on the antitumour effect of lectins has gained importance. *Pisum sativum* and *Momordica charantia* seed lectins have been reported to exert in vivo cytotoxic activity on *Ehrlich ascites* tumours (Kabir et al., 2013).

The first reports on the antitumour effect of *A. vera* lectin date from the 1980’s (Winters et al., 1981; Imanishi et al., 1981; Yagi et al., 1985). There are few recent reports on the in vivo antitumour activity of affinity chromatography-purified Aloctin (Akev et al., 2007b; Kaur et al., 2011). To date there is only one research undertaken on the in vitro cytotoxic effect of *A. vera* lectin in which HCT-15, HT-29 ve SW-620 colon cancer cells and HOP-62 lung cancer cells were used. However the mechanism of this cytotoxic effect was not elucidated (Kaur et al., 2011). To our knowledge this is the first comprehensive study, where different cancer cell lines were used for both Aloctin and AE and that apoptosis/ necrosis mechanisms were investigated. AGS stomach and Saos-2 bone cancer cells were the most sensitive cells to Aloctin cytotoxicity. The fact that the cytotoxic effect was not due to apoptosis or necrosis urge the need for further investigation on the antitumour mechanism.

In early studies, the mechanisms of the cytotoxic effects of *A. vera* lectin was related to the immunomodulatory activity (Imanishi et al., 1986; Yoshimoto et al., 1987; Winters, 1993).

Among the multiple substances found in *A. vera*, the anthraquinone glycoside AE is the most extensively studied one in regard to its antitumour effect. In several studies, AE has been reported to regress tumour growth (Imanishi et al., 1986; Yoshimoto et al., 1987; Imanishi K, Tsukuda K, Suzuki I (1981). Pharmacological studies on a plant lectin, Aloctin I. *J Fac Pharm Istanbul*, 45, 191-215. Akev N, Turkay, Can A, et al (2007a). Effect of Aloe vera leaf pulp extract on Ehrlich ascites tumours in mice. *Eur J Cancer Prev*, 16, 151-7. Akev N, Turkay G, Can A, et al (2007b). Tumour preventive effect of Aloe vera leaf pulp lectin (Aloctin I) on Ehrlich ascites tumours in mice. *Phytother Res*, 21, 1070-5. Akev N, Can A (1999). Separation and some properties of Aloe vera L. leaf pulp lectins. *Phytother Res*, 13, 489-93. Bovin NV, Gabilens H-J (1995). Polymer-immobilized carbohydrate ligands: Versatile chemical tools for biochemistry and medical sciences. *Chem Soc Rev*, 23, 413-21.

Chiu TH, Lai WW, Hsia TC, et al (2009). Aloe-emodin induces cell death through S-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 cells. *Anticancer Res*, 29, 4503-12.

Cui X-R, Takahashi K, Shimamura T, et al (2008). Preparation of 1,8-di-O-alkylaloe-emodins and 15-amino-, 15-thiocyanochrysophanol derivatives from aloe-emodin and studying their cytotoxic effects. *Chem Pharm Bull*, 56, 497-503.

Diani M (2010). Lectin Binding to Proteus Infected Rabbits Spleen Cells. Ankara University, Institute of Sciences, Department of Biology, Master Thesis, Ankara.

Falheima-Martins GV, da Silveira AL, Cavalcanti BC, et al (2012). Antiproliferative effects of lectins from Canavalia ensiformis and Canavalia brasiliensis in human leukemia cell lines. *Toxical In Vitro*, 26, 1161-9.

Gorelik E, Galli U, Jeannot M (2001). On the role of cell surface carbohydrates and their binding proteins lectins in tumor metastasis. *Cancer Metastasis Rev*, 20, 245-77.

Gribel NV, Pashinksi VG (1986). Antimetastatic properties of aloe juice. *Vopr Onkol*, 32, 38-40.

Hajtö T, Hostanska K, Berki T, et al (2005). Oncopharmacological perspectives of a plant lectin (*Viscum album Agglutinin-I*): Overview of recent results from in vitro experiments and in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.

Imanishi K, Ishiguro T, Saito H, Suzuki I (1986). Augmentation of in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.

Imanishi K, Ishiguro T, Saito H, Suzuki I (1986). Augmentation of in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.

Imanishi K, Ishiguro T, Saito H, Suzuki I (1986). Augmentation of in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.

It is important to note that the cytotoxic effect of AE was found to be higher than IM on AGS and Saos-2 cells. Therefore in Aloctin-IM combination studies, which were undertaken for the first time, the cytotoxicity was significantly enhanced in both doses tested.

We can suggest that Aloctin and aloe emodin alone or in combination are potential targets for anticancer drug research.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

References

Akev N, Can A, Sütülünar N, et al (2015). Twenty years of research on Aloe vera. *J Fac Pharm Istanbul*, 45, 191-215. Akev N, Turkay, Can A, et al (2007a). Effect of Aloe vera leaf pulp extract on Ehrlich ascites tumours in mice. *Eur J Cancer Prev*, 16, 151-7. Akev N, Turkay G, Can A, et al (2007b). Tumour preventive effect of Aloe vera leaf pulp lectin (Aloctin I) on Ehrlich ascites tumours in mice. *Phytother Res*, 21, 1070-5. Akev N, Can A (1999). Separation and some properties of Aloe vera L. leaf pulp lectins. *Phytother Res*, 13, 489-93. Bovin NV, Gabilens H-J (1995). Polymer-immobilized carbohydrate ligands: Versatile chemical tools for biochemistry and medical sciences. *Chem Soc Rev*, 23, 413-21.

Chiu TH, Lai WW, Hsia TC, et al (2009). Aloe-emodin induces cell death through S-phase arrest and caspase-dependent pathways in human tongue squamous cancer SCC-4 cells. *Anticancer Res*, 29, 4503-12.

Cui X-R, Takahashi K, Shimamura T, et al (2008). Preparation of 1,8-di-O-alkylaloe-emodins and 15-amino-, 15-thiocyan-, and 15-selenocyanochrysophanol derivatives from aloe-emodin and studying their cytotoxic effects. *Chem Pharm Bull*, 56, 497-503.

Diani M (2010). Lectin Binding to Proteus Infected Rabbits Spleen Cells. Ankara University, Institute of Sciences, Department of Biology, Master Thesis, Ankara.

Falheima-Martins GV, da Silveira AL, Cavalcanti BC, et al (2012). Antiproliferative effects of lectins from Canavalia ensiformis and Canavalia brasiliensis in human leukemia cell lines. *Toxical In Vitro*, 26, 1161-9.

Gorelik E, Galli U, Jeannot M (2001). On the role of cell surface carbohydrates and their binding proteins lectins in tumor metastasis. *Cancer Metastasis Rev*, 20, 245-77.

Gribel NV, Pashinksi VG (1986). Antimetastatic properties of aloe juice. *Vopr Onkol*, 32, 38-40.

Hajtö T, Hostanska K, Berki T, et al (2005). Oncopharmacological perspectives of a plant lectin (*Viscum album Agglutinin-I*): Overview of recent results from in vitro experiments and in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.

Imanishi K, Ishiguro T, Saito H, Suzuki I (1986). Augmentation of in vivo animal models, and their possible relevance for clinical applications. *eCAM*, 2, 59-67.
lymphokine-activated killer cell activity in vitro by Aloctin A. *Int Immunopharmacol*, **8**, 855-8.

Kabir SR, Nabi MM, Haque A, et al (2013). Pea lectin inhibits growth of Ehrlich ascites carcinoma cells by inducing apoptosis and G2/M cell cycle arrest in vivo in mice. *Phytomedicine*, **20**, 1288-96.

Kaur M, Singh J, Kamboj SS, Saxena AK (2011). Purification and characterization of a lectin from leaf pulp of Aloe vera (L.) BURM. F. *J Pharm Res*, **4**, 2441-6.

Lin J-G, Chen G-W, Li T-M, et al (2006). Aloe-emodin induces apoptosis in T24 human bladder cancer cells through the p53 dependent apoptotic pathway. *J Urol*, **175**, 343-7.

Liu B, Bian H-j, Bao J-k (2010). Plant lectins: Potential antineoplastic drugs from bench to clinic. *Cancer Lett*, **287**, 1-12.

Mahbub AA, Le Maître CL, Haywood-Small SL, et al (2013). Differential effects of polyphenols on proliferation and apoptosis in human myeloid and lymphoid leukemia cell lines. *Anti-Cancer Agent Me*, **13**, 1601-13.

Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*, **65**, 55-63.

Ozsoy N, Candoken E, Akev N (2012). Purification and antioxidant activity of Aloe vera leaf lectin. *J Fac Pharm Istanbul*, **42**, 29-39.

Seyrek K, Bildik A (2001). Lectinler. Van Vet J 12, 96-100.

Sharon N, Lis H (1972). Lectins: cell-agglutinating and sugar-specific proteins. *Science*, **177**, 949-59.

Strober W (2001). Trypan blue exclusion test of cell viability. *Curr Protoc Immunol Appendix 3, Appendix 3B. doi: 10.1002/0471142735.imaa03bs21.*

Wang H, Ng TB, Liu WK, Ooi VEC, Chang ST (1995). Isolation and characterization of two distinct lectins with antiproliferative activity from the cultured mycelia of the mushroom Tricholoma mongolicum. *Int J Pept Prot Res*, **46**, 508-13.

Winters WD (1993). Immunoreactive lectins in leaf gel from Aloe barbadensis Miller. *Phytother Res*, **7**, S23-5.

Winters WD, Benavides R, Clouse WJ (1981). Effects of Aloe extracts on human normal and tumor cells in vitro. *Econ Bot*, **35**, 89-95.

Yagi A, Machii K, Nishimura H, Shida T, Nishioka I (1985). Effect of aloe lectin on deoxyribonucleic acid synthesis in baby hamster kidney cells. *Experientia*, **41**, 669-71.

Yoshimoto R, Kondoh N, Isawa M-a, Hamaro J (1987). Plant lectin, ATF 1011, on the tumor cell surface augments tumor-specific immunity through activation of T cells specific for the lectin. *Cancer Immunol Immunother*, **25**, 25-30.

Yordanova A, Koprinarova M (2014). Is aloe-emodin a novel anticancer drug?. *Trakia J Sci*, **12**, 92-5.

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