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Effect of Fermented Soy Beverage Haelan 951 on the Viability of Human Cancer Cell Lines *in vitro*

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Abstract

The objective of this study is the assessment of the cytotoxic effect of a commercially available fermented soy beverage Haelan 951, which is a supplement widely used by cancer patients. The main ingredient of the product is soy that is rich in flavonoids, especially isoflavones. The formulation was tested for its efficacy as well as the determination of main ingredients with proliferation, viability, apoptosis assays and analytical techniques. Low concentrations of genistein and daidzein present in Haelan 951 were confirmed with HPLC, LC-MS and NMR. Cytotoxic effect against MCF7 cell line was observed with MTT, SRB and CVE at the maximum concentration (32%) after 48h of incubation. 8% Haelan 951 had apoptotic effect on all cancer cell lines tested. The formulation of the product could lead to false positive results as it was slightly soluble in all the solvents used and the viscosity was visually observed to be quite high.

Keywords: Isoflavone • Dietary supplement • Cancer • Cytotoxicity

Introduction

Cancer is one of the main causes of mortality worldwide. Predictions show that there is a 40% possibility that somebody will be diagnosed with an invasive type of cancer [1]. Over the years, researchers have increased their knowledge of cancer prognosis, diagnosis and progression at genetic, epigenetic, molecular and cellular levels [2]. As a result, researchers have important tools for effective therapeutics to fight cancer and opportunities to combine different types of treatment in order the patient to achieve a better response [3].

Chemotherapy has been widely used to treat various types of cancer but the outcome of the administration of that type of treatment is severe adverse effects. Researchers are looking for natural-based alternatives that may have less toxicity than chemotherapy [4]. Purified organic compounds extracted from natural sources have been used for the development of many FDA approved drugs. There are many *in vitro* studies proving the anti-tumor effect of many natural products and that is the reason that they are considered as promising candidates for cancer treatment [5].

Isoflavones are polyphenolic plant-derived compounds with estrogenic and antiestrogenic activity. They function as antioxidants, deregulate cell cycle progression, induce apoptosis and inhibit tumor invasion. Soybean is a very rich source of isoflavones, containing the glycosides genistin, daidzin and others [6]. Fermentation of soy results in the formation of isoflavone aglycones such as the most abundant and active genistein, along with glycitein and daidzein. Aglycones, when administrated orally, they are absorbed rapidly and in greater quantities [7].

Haelan 951 is a commercial fermented soy product that is consumed by many cancer patients [8]. It is supposed to contain genistein and daidzein and there are important health claims stated by the manufacturers. It is suggested as “an immune boosting nutritional aid with proven history of improving health and quality of life”.

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The health effects of natural products, including isoflavones, depend on the consumed amount and their bioavailability. Low bioavailability is very commonly observed when consuming natural products and that is the basic reason for showing low efficacy. Overconsumption may be related to safety concerns due to high levels of those substances in the organism [9].

Patients have to be very careful when making the decision of consuming that type of products as there is not stringent regulation on the commercialization and use of dietary supplements, resulting in distribution of products that have not passed quality control [10]. Thus, this study focuses on the anticancer effects of Haelan 951 supplement. We performed solubility test and LC-MS analysis for the detection of the substances that were present in the product. Cytotoxicity against cancer cells was determined with colorimetric assays that were representing the viability of cancer cells. Moreover, we investigated the apoptosis of cancer cells after the treatment with various concentration of Haelan 951.

The scope of this study was the evaluation of the effectiveness of the commercially available beverage that is widely used by cancer patients. Firstly, the assessment of the formulation of the product that was appropriate for consumption and then the determination of any anticancer effect it might have against different specific human cancer cell lines.

Material and Methods

Solubility

Ethyl alcohol, methyl alcohol and dimethyl formamide were obtained from Sigma-Aldrich (Taufkirchen, Germany) and water for injection from BIOSER (Trikala, Greece). Tests were performed by adding 0.5mg of freeze dried Haelan 951 (Haelan Products, Inc., WA, U.S.A., Batch # 4180915) to test tubes containing 0.1ml of solvent and mixing by using a Stuart vortex mixer SA8 (Staffordshire, United Kingdom) for 5min at 2500rpm and an mrc sonication bath AC-150H (Essex, United Kingdom) for 5min. The quantity of each solvent was increased until final volume of 1ml and solubility was assessed visually.

NMR analysis

Samples were prepared by adding 5mg of freeze dried Haelan 951 to 0.5ml of deuterated solvent (DMSO-d6 and methanol-d4, Eurisotop, MA, U.S.A.) in 5mm NMR tubes. Two of them were analyzed immediately and two were left for a five day extraction. 1H-NMR and DOSY spectra were obtained by operating a 400 MHz Bruker Avance spectrometer (AV-III-HD, 400, Rheinstetten, Germany). The parameters used to execute the NMR experiments were: Pulse angle, 30°; pulse width, 41.6 μs; data points 96152; and number of

scans, 64; acquisition time (AQ), 3.999 s; spectral width, 12019.23 Hz. A line-broadening factor of 0.1 Hz was applied to FIDs before Fourier transformation, and the repetition delay was 60 s. All chemical shifts were reported in parts per million (ppm) according to the deuterated solvents used. Phase and baseline distortions were also applied to automatically correct all of the spectra, using TOPSPIN (version 3.5pl5, Bruker Biospin, Spring, TX, USA). For DOSY 1H NMR, stimulated echo bipolar gradient pulse experiments were used with a pulse delay of 5 ms after each gradient, a pulse-field gradient length of 1 ms and a diffusion delay of 100 ms.

All data were processed with TOPSPIN (version 3.5pl5, Bruker Biospin, spring, TX, USA) software. The processing parameters were 1024 points along the Laplace spectrum diffusion axis and 20000 MaxEnt iterations. In the DOSY spectra the horizontal axis represents the chemical shifts (ppm) and the vertical axis the diffusion coefficients (μm2s−1).

HPLC and LC-MS analysis

Genistein, glycitein and daidzein analytical standards were purchased from Sigma-Aldrich (Louis, MO, USA). Methyl cyanide and water for HPLC were also obtained from Sigma-Aldrich (Taufkirchen, Germany) and glacial acetic acid was purchased from Merck KGaA (Darmstadt, Germany). LC-MS grade water and acetonitrile were purchased from Fisher Scientific (Pittsburgh, USA). One bottle (8 fl. oz. (236ml) of Haelan 951 was purchased from Haelan Products Inc. (WA, USA), (Batch # 4180915).

For HPLC and LC-MS analysis, two types of samples were prepared. One sample was prepared without any treatment for LC-MS analysis (untreated sample) and one was hydrolyzed with the use of enzymes to release any conjugated isoflavones (treated sample). Two different vials containing 1ml of Haelan 951 were lyophilized. One was extracted with 5ml of methanol-water (75:25 v/v) in a sonicator bath for 20min and leaving it overnight before filtration to remove insoluble matter. Solvent was evaporated and 40units of 1,4-(1,3:1,4)-β–D-Glucan 4-glucanohydrolase (Sigma-Aldrich, Taufkirchen, Germany) in 2ml of 0.1M aqueous ammonium acetate solution, pH=5.0, was added. The sample went through sonication and overnight incubation at 37°C before being analyzed. The other sample was prepared by adding 5ml of methanol, sonicating for 20min and leaving it overnight to allow the extraction of isoflavones before filtrating through a 0.45 μm nylon filter and analyzed further for the assay. Samples of analytical standards of genistein, glycitein and daidzein of the same concentration were prepared in methanol.

HPLC analysis was performed using an Agilent 1260 Infinity Series equipped with a MWD detector (Agilent Technologies Inc., Richardson, TX, USA). Open LAB Chemstation was used to integrate and analyze the chromatograms (version M8301AA, Revision C.01.07 Agilent Technologies Inc., Richardson, TX, USA).

Isoflavones were analyzed by using a column (Zorbax Eclipse RP C18 reverse-phase, 250 mm x 4.6 mm I.D., 5 μm, Agilent, Santa Clara, CA, and USA) retained at 40 °C. The mobile phases used were: (A) water, (B) methyl cyanide and (C) glacial acetic acid. (A), (B) and (C) were mixed at a 67.5: 25.0:7.5 (v/v) ratios. Flow rate was 1.5 ml/min, injection volume was 10 μL and ultraviolet (UV) detection was carried out at 260 nm. The time period of analysis was 30min.

Analysis with LC-MS (1260 Infinity Series HPLC, 6120 Quadrupole MSD, Agilent Technologies Inc., Richardson, TX, USA) was carried out using a reverse phase column, Zorbax RX-C8 (5 μm, 250 mm × 4.6 mm, Agilent Technologies, Santa Clara, CA, USA) maintained at 40 °C. Open LAB Chemstation was used to integrate and analyze the chromatograms (version M8301AA, Revision C.01.07 Agilent Technologies Inc., Richardson, TX, USA). The mobile phases used were: (A) water, (B) methyl cyanide and (C) glacial acetic acid and were mixed at a ratio of 67.5: 25.0:7.5 (v/v). Flow rate was 0.7 mL/min and the analysis time 30 min. The injection volume was 10 μL and ultraviolet (UV) detection was carried out at 260nm. Mass spectrometry was performed with a 6120 Series Quadrupole System (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray ionization source (ESI). The mass detector parameters were set as follows: nitrogen as drying gas with a flow rate of 12L/min at 350oC, nebulizer pressure at 35psi and capillary voltage at 3kV, negative mode operation, scan analysis and a 100–1000 m/z scan range.

Cell culture

Four different commercial cancer cell lines that were purchased from ECACC (European Collection of Authenticated Cell Cultures) (Salisbury, UK) were used in the present study. MCF7 human breast adenocarcinoma cells (luminal type) (ECACC 86012803) were cultured in RPMI 1640 supplemented with 10% FBS, 2% L-Glutamine and 2% NEAA. MDA-MB-231 human breast adenocarcinoma cells (triple negative) (ECACC 92020424) were cultured in RPMI 1640 supplemented with 15% FBS and 2% L-Glutamine. HCT116 human colorectal carcinoma cells (ECACC 91091005) were cultured in DMEM supplemented with 10% FBS and 2% L-Glutamine. COLO699N human lung cancer cells (ECACC 93052608) were cultured in RPMI 1640 supplemented with 10% FBS and 2% L-Glutamine. RPMI 1640 Catalog#R0883 and L-glutamine Catalog#G7513-100ML were obtained from Sigma-Aldrich, Darmstadt, Germany and FBS Catalog#FB-1001/500 was obtained from Bios era, Nuaille, France. MEM Non-essential amino acids (NEAA) Catalog#M7145-100ML and DMEM (Dulbecco's Modified Eagle Medium) Catalog#D5546 were purchased from Sigma-Aldrich, Darmstadt, Germany. Cell cultures were grown in a humidified incubator at 37oC and 5% CO2 and passaged when cells reached 80% confluence.

Exposure to increasing concentrations of Haelan 951

Viable cells were seeded at a density of 2 x 104 (200μl/well) in 96-well plate and incubated at 37oC and 5% CO2 for 24h to form a cell monolayer. After 24h of incubation, supernatant on the monolayer was discarded and 200μl of medium and varying concentrations (1%, 2%, 4%, 8%, 16% and 32%) of the Haelan 951 beverage were added and incubated for 24, 48 and 72h time points.

Cell proliferation assay

MTT assay was employed to measure the proliferation rate based on the cleavage of the tetrazolium salt in the metabolic active cells only, leading to the formation of formazan crystals. After the specific time points, 20μl of 5mg/ml MTT Catalog#M2128 (Sigma-Aldrich, Darmstadt, Germany) in PBS Catalog#P3813 (Sigma-Aldrich, Darmstadt, Germany) was added to each well and incubated for 3h at 37oC and 5% CO2. Supernatants were discarded and 100μl of DMSO Catalog#445103 (Carlo Erbo Reagents, Barcelona, Spain) was added and the plates were incubated for 5 min 37oC and 5% CO2 to solubilize the formazan crystals and absorbance was measured at 560nm and the reference wavelength was at 605nm.

Cellular viability analysis

Viability of cells was assessed with two colorimetric assays, SRB and CVE, which are used for the determination of cellular protein content. For the SRB assay, at the specific time points cells were fixed by adding 100μl trichloroacetic acid Catalog#91228 (Sigma-Aldrich, Darmstadt, Germany) and with incubation at 4oC for 1h. Next, 100μl of SRB solution Catalog#341738 (Sigma-Aldrich, Darmstadt, Germany) was added in the wells for 30 min at room temperature. After that, supernatant was discarded, cells were rinsed three times with 1% glacial acetic acid Catalog#1.00063.1011 (Merck, Darmstadt, Germany) and were left to air dry. Finally, the dye was solubilized with 200μl of 10mM Tris-base pH 10.5 Catalog#T6791 (Sigma-Aldrich, Darmstadt, Germany) and absorbance was measured at 570nm and the reference wavelength was at 605nm.

For the CVE assay, at the specific time points, supernatant was removed and cells were fixed with 100μl 10% formalin for 20 min. Formalin was discarded and cells were left to air dry. Next, cells were dyed with 100μl of 0.25% aqueous crystal violet solution Catalog#HT901 (Sigma-Aldrich, Darmstadt, Germany) and left for 10 min at room temperature. Then, supernatant was discarded and cells were rinsed twice with 100μl WFI (water for injection) and were left to air dry. The dye was solubilized with 100μl of 33% glacial acetic acid Catalog#1.00063.1011 (Merck, Darmstadt, Germany) and absorbance was measured at 570nm and the reference wavelength was at 605nm.

Statistical analysis

The experiments of cellular proliferation and cellular viability determination were performed in triplicates. The average absorbance was calculated for each triplicate. Subsequently, the sample measurements were corrected for the measurement of the blank. The differences in the mean were determined with one sample t-test by comparing the treated samples with the untreated controls. A statistically significant difference was considered with p values <0.05. Results were calculated using the Microsoft Excel 2016.

The measurements from the proliferation and viability assays were used to determine the IC50 values by using the Microsoft Excel 2016.

Detection and quantification of apoptosis

The detection of cell apoptosis was performed with the Annexin V PE Detection Kit (Becton Dickinson, Heidelberg, Germany). It was used to distinguish cells that were viable, early apoptotic, late apoptotic or dead. The assay was performed according to the manufacturer's instructions. Analysis of the cells was performed on a FACS Calibur (Becton Dickinson, USA) and FSC 6 Express software. Ten thousand events were measured per sample.

Results

Solubility test

The solubility was assessed visually to assist with further analysis. Solubility was observed in several polar solvents and the results are presented in Table 1.

NMR analysis

It is known that isoflavones are soluble in methanol and DMSO. These two solvents were chosen run a 1H and DOSY NMR on Haelan in order to observe better its solubility and contents.

No new peaks were observed before Figures 1 and 3 and after Figures 2 and 4 the 5-day extraction in both solvents, which means that extraction has little effect on Haelan's ingredients. Peaks in the 6-9 ppm region confirm the presence of isoflavones. Peaks in the 1-5 ppm region confirm the presence of saponins and peaks in the 0.6-1.1 ppm region confirm the presence of phytosterols. However, the small-intensity peaks of isoflavones show that their amount is significantly smaller compared to the other ingredients.

HPLC and LC-MS analysis

Two types of Haelan 951 samples were analyzed by a validated LC-MS method and 3 isoflavones were identified and quantified. One sample was directly prepared for LC-MS analysis (untreated sample) and one went through enzymatic hydrolysis of the glycoside conjugates (hydrolyzed sample). Although isoflavones (genistein, daidzein, & genistein) are considered the main active ingredients in Haelan formula (fermented soy beverage) and it is stated that they exist in "carefully calculated levels", their names and quantities are not mentioned on the label. Quantitative analysis shows that the concentrations of Genistein, Daidzein and Glycitein, both in free and glycoside conjugate form, are: 195, 152 and 16 mm (Table 2).

Effects of different Haelan 951 concentrations on the proliferation of cancer cell lines

To examine the effect of Haelan 951 on cellular proliferation, the mitochondrial activity was measured with MTT assay. According to the results from the specific assay, IC50 values were calculated for each human cancer cell line (Table 3). IC50 was calculated by using a free online tool. The concentration of Haelan 951 that appeared to have an effect on the growth of MCF7 and COLO699N cell lines was close to the highest concentration of 32% that was used in the assay. It was 31.3% and 31.6% for MCF7 at 48h and 72h incubation time, respectively. For COLO699N, IC50 values were 21.3%, 24.1% and 30.7% at 24h, 48h and 72h incubation time, respectively. For the rest of the cell lines, the effect of the different concentrations of Haelan 951 at the different incubation times was very similar to the untreated cells control and the IC50 value could not be calculated.

Effects of different Haelan 951 concentrations on the viability of cancer cell lines

The human cancer cell lines MCF7, MDA-MB-231, HCT116 and COLO699N were treated with Haelan 951 at concentrations ranging from 2% to 32% for 24h, 48h and 72h. Cellular viability was determined with two colorimetric assays that were measuring the protein content of viable cells, SRB and CVE (Tables 4 and 5). There was no significant effect observed at any of the cell lines as the IC50 values revealed that very high concentrations of the Haelan 951 were needed in order to inhibit *in vitro* the biological process of the cancer cells by 50%. According to both assays, it appeared that 32% that was the maximum concentration tested, had an effect on MCF7 cell line at 48h of incubation. The two lowest concentrations observed were at 48h of incubation with Haelan 951 at COLO699N and HCT116 cell lines and it was 25.7% and 29%, respectively, with the SRB assay.

Effect of 8% Haelan 951 on apoptosis of cancer cell lines

To determine whether Haelan 951 causes apoptotic cell death, cells were stained with Annexin V-PE and 7-AAD and analyzed with flow cytometry after 24h, 48h and 72h of incubation. Viable cells were double negative, apoptotic cells were positive only to Annexin V, late apoptotic cells were double positive and dead cells were positive only to 7-AAD. The range of concentrations was the same as the previous assays, 2% to 32%, but the concentration of 8%

SOLVENT	VOLUME	OBSERVATION
Ethanol	0.1-1 ml	Slightly soluble
Methanol	0.1-1 ml	Slightly soluble
Dimethylsulfoxide	0.1-1 ml	Slightly soluble
Water for Injection	0.1-1 ml	Slightly soluble

Table 1. Solubility assessment of Haelan 951 and results.

	CONCENTRATION (mM)		
ISOFLAVONE	Untreated Haelan sample	Hydrolysed Haelan sample	Total
Genistein	13	182	195
Daidzein	31	121	152
Glycitein	2	14	16

Table 2. Concentration of is flavones in free and glycoside conjugate form.

Haelan 951 MTT	MCF7	COLO699N
24h	NS	21.30% (p=0.002)
48h	31.30% (p=0.001)	24.10% (p=0.001)
72h	31.60% (p=0.000025)	30.70% (p=0.01)

Table 3. IC50 values with MTT assay ("NS" - No Significant, no effect on MDA-MB-231 and HCT116).

Haelan 951 SRB	MCF7	MDA-MB-231	HCT116	COLO699N
24h	48.40% (p=0.006)	NS	NS	78.40% (p=0.04)
48h	32.40% (p=0.002)	55.90% (p=0.02)	29.00% (p=0.0006)	25.70% (p=0.0002)
72h	53.90% (p=0.04)	105.90% (p=0.02)	NS	40.20% (p=0.01)

Table 4. IC50 values with SRB assay ("NS" - No Significant).

Haelan 951 CVE	MCF7	MDA-MB-231	HCT116	COLO699N
48h	NS	NS	31.20% (p=0.004)	40.20% (p=0.01)
72h	73.40% (p=0.01)	37.10% (p=0.002)	125.70% (p=0.009)	NS

Table 5. IC50 values with CVE assay (no effect of the Haelan 951 at 24h).

8% Haelan 951 24h	Viable	Apoptotic	Late Apoptotic	Dead
MCF7	63.99 (45.96)	13.47 (43.22)	15.93 (10.40)	6.91 (0.43)
MDA-MB-231	14.71 (22.66)	19.79 (31.41)	63.08 (44.70)	2.42 (1.22)
HCT116	9.96 (18.86)	36.54 (62.84)	51.06 (18.13)	2.45 (0.16)
COLO699N	14.77 (50.82)	42.88 (34.34)	41.08 (14.51)	1.26 (0.33)

Table 6. Apoptosis results for the human cancer cell lines after treatment with 8% Haelan for 24h (values in brackets represent the values of the untreated control).

32% Haelan 951 48h	Viable	Apoptotic	Late Apoptotic	Dead
MCF7	45.74 (76.78)	36.17 (21.85)	18.09 (1.35)	0.00 (0.01)

Table 7. Apoptosis results for the human cancer cell line MCF7 treated with 32% Haelan for 48h (values in brackets represent the values of the untreated control).

appeared to be the most significant in all cell lines at 24h (Table 6). The results revealed that the numbers of late apoptotic and dead cells were increased in all cell lines. The number of viable cells was reduced to the half of the untreated control in the three cell lines, MDA-MB-231, HCT116 and COLO699N.

Effect of 32% Haelan 951 on apoptosis in MCF7 at 48h

The two viability assays (SRB, CVE) and the proliferation assay (MTT) showed that the maximum concentration of Haelan 951 had an effect on the proliferation and viability of MCF7 cell line at 48h. Flow cytometry with the Annexin V and 7-AAD was performed at the specific concentration and incubation time in order to determine the effect on the apoptosis of the cells (Table 7). The number of viable cells was reduced to 45.74%, while the number of apoptotic cells was increased to 36.17%. The higher increase was observed at the number of late apoptotic cells that reached the 18.09%.

Discussion

In the present work, we investigated the effect of the fermented soy beverage Haelan 951 on the proliferation, viability and apoptosis in human cancer cell lines. Haelan 951 contains nutrients such as selenium, zinc, vitamins, fatty acids and flavonoids. The two major components are the isoflavone genistein and daidzein [11]. Our results suggest that Haelan 951 contains saponins, phytosterols and isoflavone. The concentration of isoflavones that are present in the product is not very high and that might have an effect on the efficacy of the product.

Both genistein and daidzein that were present in Haelan 951 have been reported to increase the proliferation of MCF7 cell line by increasing DNA synthesis in low concentrations but not the proliferation of the hormone insensitive breast cancer cell line MDA-MB-231 [12-14]. When the concentrations are low the inhibitory effect on cellular proliferation was enhanced, leading to significant growth arrest [8].

When human cancer cell lines were exposed to higher concentrations of Haelan 951, there was no induction of growth arrest but strong cytotoxic effects [15]. High concentrations of genistein and daidzein have also been reported to reduce the viability of cells from various cancer types by causing damage to the cells [16]. The result that the proliferation of MCF7 and COLO699N was reduced even in the highest concentration of Haelan 951, represent that those two cancer cell lines might be more sensitive to the specific soy beverage than MDA-MB-231 and HCT116.

Concerning the apoptosis inducing activity, the results revealed that 8% of Haelan 951 was effective in inducing apoptosis in all cell lines tested. Human lung cancer cell line COLO699N seemed to be more sensitive to 8% Haelan 951 while MCF7 showed the lowest sensitivity to 8% Haelan 951 at 24h. The cells possibly entered the apoptotic pathway in a caspase-dependent manner as it had been reported previously [8].

The involvements of isoflavone, genistein and daidzein, in the molecular pathways that are related to cell growth arrest and apoptosis have been explained. Inductions of DNA damage by inhibition of tyrosine kinases and topoisomerase II that result in activation of p53 tumor-suppressor protein-dependent pathways [17]. Low stress induces low expression of p53 resulting in cell cycle arrest and in turn survival genes are expressed in order to repair any cell damage [18]. The effect of flavonoids on the cell cycle is depending on the concentration, the exposure time and the cancer cell line. High DNA damage leads to higher expression of p53 resulting in apoptosis to prevent passing the genetic defects to new cell generations [19].

In summary, the present study revealed that the commercially available fermented soy beverage Haelan 951 contains flavonoids among other nutrients but the concentration of isoflavone, genistein, daidzein and Glycitein, was really low. The results were obtained from assays concerning the effect of various concentrations and incubation time of the product on the proliferation, viability and apoptosis of four different human cancer cell lines. The four cell lines showed different responses after exposure to the Haelan 951, with MCF7 and COLO699N showing greater sensitivity than MDA-MB-231 and HCT116. According to the above results, there was a specific concentration (32%) of Haelan 951 that showed cytotoxic effect at a specific cancer cell line (MCF7 human adenocarcinoma) at a specific time point (48h), but this effect could be attributed to the high viscosity the supplement presents at this concentration leading to false positive results. Flow cytometry results revealed that Haelan 951 had an effect in the viability of all human cell lines at lower concentrations (8% and 16%) than in the other assays.

Conclusion

The beverage Haelan 951 contains isoflavone in low concentrations among other nutrients. The cytotoxic effect against a human hormone sensitive breast cancer, a human triple negative breast cancer, a human colorectal carcinoma and a human lung cancer cell line was not very strong. The IC50 values of Haelan 951against all human cancer cell lines was high indicating that *in vitro* the efficacy of the product was limited. Higher concentrations of isoflavone in the product could have better efficacy.

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Disclosure

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