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ORIGINAL ARTICLE

The Effect of Cycloartane-Type of Saponins from *Astragalus* Species on the Proliferation of MCF-7 and MDA-MB-231 Breast Cancer Cells

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OBJECTIVE

Saponins are the main components of *Astragalus* species. In this study, different doses of saponins obtained from *Astragalus* species were applied to MCF-7 and MDA-MB-231 breast cancer cell lines, and the cell proliferation, cytotoxicity, and apoptotic effects were investigated.

METHODS

Five different cycloartane-type saponins (Astragaloside IV, Cyclocanthoside E, Astrasieversianin X, Macrophylosaponins B and D) were incubated with MCF-7 and MDA-MB-231 cells for 24, 48, and 72 h. Cell cytotoxicity activity of saponins on cell lines was determined by cell counting kit 8. For apoptosis analysis, TUNEL Assay Kit was used.

RESULTS

Significant changes in cytotoxicity were obtained at concentrations of 10 μM, 100 μM and 200 μM for 24 h, at concentrations of 100 μM and 150 μM for 48 h, and at concentrations of 10 μM and 100 μM for 72 h in the MDA-MB-231 cells, respectively. In MCF-7 cells, no significant changes in the cell cytotoxicity were obtained between the control and administered concentrations for 24 h but significant changes were obtained at all concentrations (10 μM, 100 μM, 150 μM, 200 μM) for 48 h and at concentration of 100 μM for 72 h. There was a significant change in the apoptosis analysis for the MCF-7 cells at concentrations of 10 μM and 100 μM for 48 h.

CONCLUSION

All in all, this study suggests that low-dose saponin glycosides decreased cell viability of breast cancer cell and increased apoptosis in MCF-7 cells.

Keywords: *Astragalus* species; cancer; cycloartane-type saponins; MCF-7; MDA-MB-231.

INTRODUCTION

Breast cancer is the most important health problem frequently seen in women, and according to The Global Cancer Observatory 2020 data 2.3 million new breast cancer cases and 685,000 breast cancer-related demise were detected around the world.[1] In cancer treatment, various herbal products are used as supplements, to re-

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duce the toxicity of chemotherapy and radiotherapy, and to relieve pain caused by cancer.[2] The application of some food or bioactive compounds positively supports the course of the disease and reveals physiological changes to improve the life standards of cancer patients.[3,4]

The *Astragalus* L. species, which belongs to the Fabaceae (legume) family, has attracted great interest in many different societies, especially Chinese and Turkish, since ancient times. Raw extracts and isolated components of *Astragalus* species, which is known to be represented by approximately 440 species in the flora of Turkey, showed anti-inflammatory, antioxidative, anticancer, and antiviral activities. Cycloartane-type saponins and polysaccharides are the most important components of *Astragalus*.[5] *Astragalus* polysaccharides have been widely used in studies of cancer therapy due to their low toxicity, immunomodulatory, and anticancer properties. Saponins, also known as triterpenoid glycosides, are also used as pharmaceutical or nutraceutical agents.[6] The apoptosis is induced in tumor cells by use of saponins. Apoptosis in tumor cells helps to reduce side effects in cancer patients by suppressing necrosis.[7] *Astragalus* species are an important source for saponins, with Astragaloside IV being the most studied saponin glycoside.[8] In a study, MCF-7 ve MDA-MB-231 cancer lines growth and viability is limited with the use of the Astragaloside IV.[9]

In this study, five different (astragaloside IV, cyclocanthoside E, astrasieversianin X, macrophylosaponins B and D) cycloartane-type saponins isolated from *Astragalus* species, whose effects on breast cancer have not been studied, were investigated at several doses on MCF-7 and MDA-MB-231 breast cancer cell lines and their cytotoxic, antiproliferative, and antiapoptotic activities were investigated.

MATERIALS AND METHODS

Saponin Extraction and Isolation

Astragaloside IV, Astrasieversianin X, Cyclocanthoside E were isolated from *Astragalus melanophrurius Boiss*. [10] and Macrophyllosaponins B and D from *Astragalus oleifolius* DC. (section of genus: *Macrophyllium*). [11] The extraction, isolation, and structure elucidation of these cycloartane type glycosides were previously explained.[10,11]

Cell Preparation

MCF-7 and MDA-MB-231 (American Type Culture Collection, Rockville, Maryland, USA) cell lines are generally used in breast cancer cell studies. MCF-7 cells have weak invasion and migration capacity when compared to MDA-MB-231 cells.[12] Cell lines were grown in DMEM supplemented with 10% fetal bovine serum as a growth medium. Cells were grown in T-25-cm^2 cell culture flask to 80% confluency at 37°C, in a 5% CO_2 and humidified incubator. Cell medium was replaced every day and cells routinely subcultured.

Cell Viability/Cytotoxicity Assays

To evaluate the cytotoxic activity of saponins from *Astragalus* species on the proliferation of breast cancer cells, the TEBUBIO cell counting kit 8 (CCK 8) assay was applied in accordance with the kit procedure. 100 µL cell were placed in 96-well plates based on cell count. After 24 h of incubation, different concentrations of *Astragalus* extracts (10 µM, 100 µM, 150 µM, 200 µM) were added to cell for 24 h, 48 h, and 72 h. Then cells, with the use of CCK-8 product, were incubated for 4 h. The absorbances are determined at 450 nm wavelength in an absorbance microplate reader.

Apoptosis Detection by TUNEL Method

Apoptosis detection with the effect of saponins was evaluated by the Apoptag Plus Peroxidase in Situ (Sigma-Aldrich, USA) kit on the cancer cells. Cells were proliferated on slides and different types of *Astragalus* saponins, at concentrations of 10 µM and 100 µM, were added based on the IC_{50} value from the cell viability assay and stained according to the kit procedure for 24–48 h. TUNEL-positive stained cells in groups treated with *Astragalus* saponins at different times and concentrations were counted in 10 fields with ×40 objective magnification. This method was done over one repetition for each Saponins.

Statistical Analysis

A significant difference between groups was evaluated by Kruskal–Wallis test using PAWS STATISTIC 18. Intra-group significance was evaluated with the Mann–Whitney U test. The viability/cytotoxicity results of the cells for the different saponins concentrations at different time intervals were evaluated using GRAPHPAD PRISM SOFTWARE (version 8). $P<0.05$ values were statistically significant.

RESULTS

Cytotoxicity Results

Breast cancer cell proliferation was suppressed with the saponins from *Astragalus* species. CCK-8 kit was used to determine cytotoxic effect of saponin extracts. MCF-7 and MDA-MB-231 cells were treated with different

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Table 1 IC_{50} (μM) values of saponins in MDA-MB-231 breast cancer cell line

Exposure time	Astragaloside IV	Astrasieversianin X	Macrophylosaponin-D	Macrophylosaponin-B	Cyclocanthoside E
24 h	75.60	16.00	24.00	3.53	23.89
48 h	15.00	10.00	12.80	7.53	3.878
72 h	88.9	20.25	51.70	3.27	69.88

MDA-MB-231: Isolated at M D Anderson from a pleural effusion of a patient with invasive ductal carcinoma

Table 2 IC_{50} (μM) values of saponins in MCF-7 breast cancer cell line

Exposure time	Astragaloside IV	Astrasieversianin X	Macrophylosaponin-D	Macrophylosaponin-B	Cyclocanthoside E
24 h	15.87	24.34	52.76	13.5	11.8
48 h	12.7	13.15	2.89	12.97	12.85
72 h	32.41	61.27	3.89	20.77	54.74

MCF-7: Michigan Cancer Foundation-7

concentration of saponins for 24, 48 and 72 h to evaluate the cytotoxicity of saponins. IC_{50} values of each saponins in MCF-7 and MDA-MB-231 cells are shown in Tables 1 and 2. For MCF-7 cell line after 24h, the mean difference was not significant at 95% confidence level (CI) between the control absorbency value and absorbency values obtained at 200 μM, 150 μM, 100 μM, and 10 μM saponins concentrations. However, in all concentrations cell viability decreased significantly when compared with the control group after 48 h exposure. The mean difference was not significant at the 95% CI between the control absorbency value and absorbency values obtained at 200 μM, 150 μM and 10 μM saponins concentrations after 72 h exposure. When the saponin concentration decreased from 200 μM to 100 μM, absorbency decreased so the alive cell number decreased.

For the MDA-MB-231 cell line after 24h, the mean difference was not significant at 95% confidence level (CI) between the control absorbency value and absorbency values obtained at 200 μM and 150 μM saponins concentrations. However, cell viability decreased significantly at 10 μM and 100 μM concentrations and the control group after 24 h exposure. Furthermore, after 48 h exposure, significance was observed at 100 μM and 150 μM concentrations and the control group. The mean difference was significant at the 95% CI between the control absorbency value and absorbency values obtained at 10 μM and 100 μM concentrations of saponins after 72h exposure. The absorbency value at the lowest concentrations of saponins was the lowest when compared to other concentrations. Hence, the lowest concentrations have the highest cytotoxic effect on both MCF-7 and MAD-MB-231 breast cancer cells. Breast cancer cell growth was significantly inhibited in both cell lines depending on dose and time within 24-48-72 h ($p<0.05$) (Figs. 1, 2). Also with the morphological examination, these results were approved. As a result of the agent interaction, it was observed that a portion of cells died in Cyclocanthoside E, Astrasieversianin X and Astragaloside IV agents (Fig. 3).

TUNEL Analysis

When p values of the saponin species used in the MDA-MB-231 cell line were compared with the control, there was no statistically significant. A strong activity of the cycloartane-type glycosides on the MCF-7 cell line was observed. 10 μM and 100 μM of glycoside samples were added to breast cancer cells for 48 h showing an increase in the number of cell apoptosis (Fig. 4). When the p values obtained from all saponins (Cyclocanthoside E, Astragaloside IV, Macrophilosaponin B and D, Astrasieversianin X) used in the MCF-7 cell line were compared with the control, statistically significant difference observed; p values were found as 0.013, 0.013, 0.014, 0.047, and 0.013, respectively.

DISCUSSION

Breast cancer is the most commonly diagnosed cancer type among woman globally. New treatment strategies and earlier detection decreases death rates.[13] Side effects caused by traditional treatment methods, such as drugs

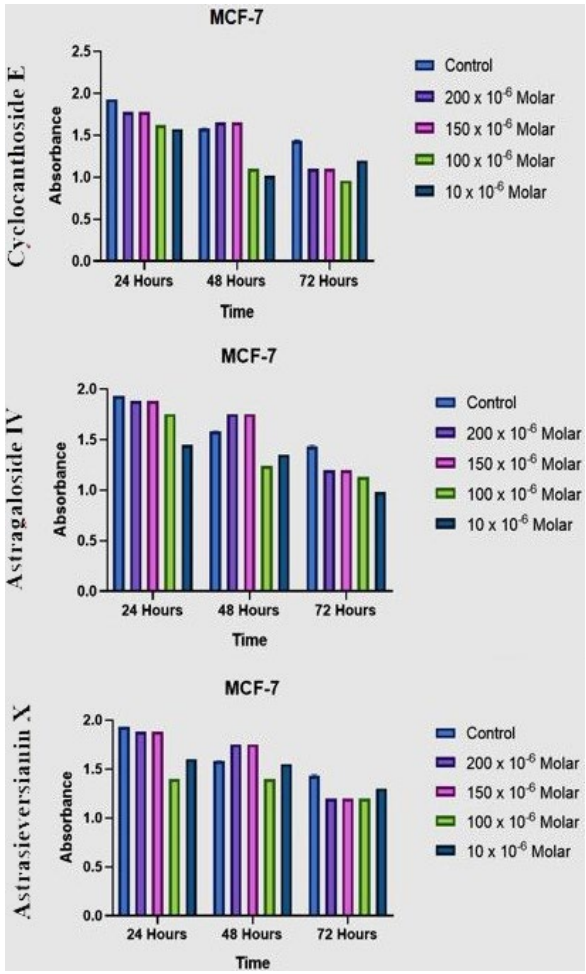

Fig. 1. CCK8 test values of *Astragalus* saponin extracts to MCF-7 cells at different concentrations and 24-48-72-time intervals.
MCF-7: Michigan Cancer Foundation-7; CCK8: cell counting kit 8.

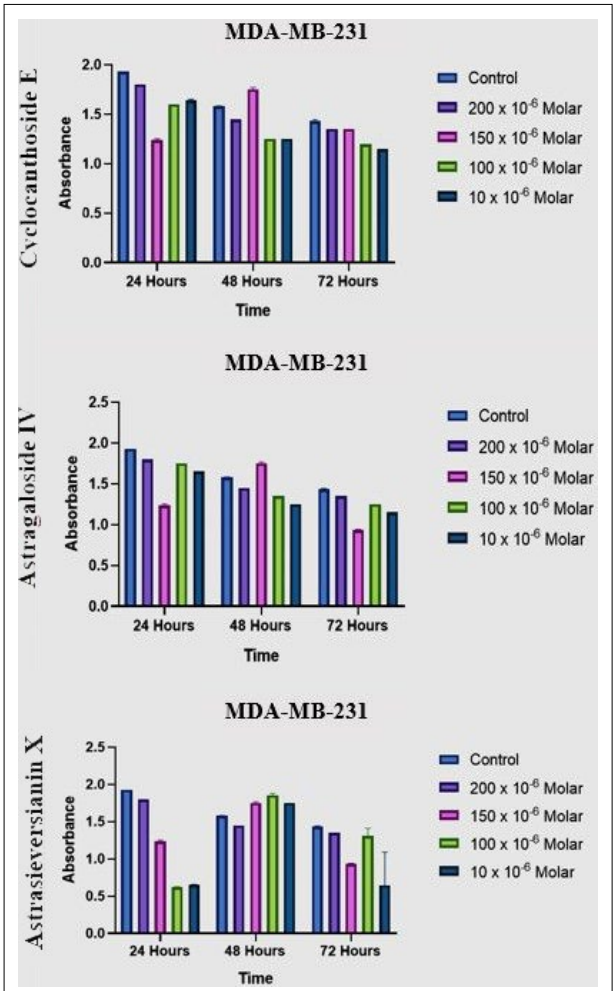

Fig. 2. CCK8 test values of *Astragalus* saponin extracts to MDA-MB-231 cells at different concentrations and at 24-48-72-time intervals.
MDA-MB-231: Isolated at M D Anderson from a pleural effusion of a patient with invasive ductal carcinoma; CCK8: cell counting kit 8.

toxicity and the repetition of neoplasm due to aggressive behaviour create significant problems in breast cancer patients. There is an alternative treatment strategies getting greatest interest in today's world. Products obtained from the whole or some parts of the plants are used as a supplement in treatment.[14] Natural compounds such as quercetin, curcumin, soy isoflavones, and lentinan are used as potential chemopreventive agents.[15–17] Resveratrol reduces the adverse effect of cancer drugs.[18] The effects of curcumin for chemoprevention have been reported according to many molecular mechanisms, such as inducing apoptosis and reactive oxidative species scavenging.[19] Studies indicated that cancer risk-reducing with the use of ginseng in humans. The active compound of ginseng reactivates natural killer cells that are disrupted during chemotherapy and radiotherapy, increases antibody formation by inducing macrophages.[20] Saponins have anti-cancer activities through by targeting many cancer-related pathways. It targets cell cycle arrest, and induce apoptosis, ER stress activation, and migration inhibition. Ginsenosides are an active constituent of Ginseng and they consist steroidal saponins, protopanaxadiols, and protopanaxatriols. Ginsenosides suppresses cancer cells growth, promotes tumor cell cycle arrest and apoptosis, induces autophagy and necrosis, and also inhibits invasion and metastasis of cancer cells.[21]

Saponins are commonly found in flowering plants (Angiospermae) of medicinally important plants species such as *Aesculus hippocastanum* L., Gycyrrhiza

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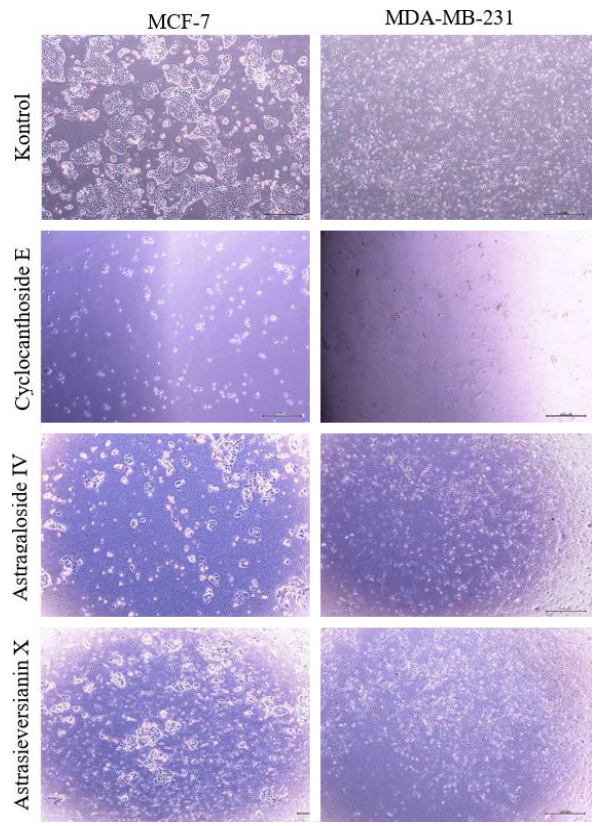

Fig. 3. Microscopic images obtained as a result of CCK-8 cytotoxicity test.
CCK8: cell counting kit 8.

inflata Bat., *Panax ginseng* C.A.Mey., *Astragalus* L., *Bupleurum chinense* DC., *Primula vulgaris* Huds., *Cyclaminos Heldr*. and *Hedera helix* L. from the subdivision *Dicotyledonae* and *Dioscorea*, *Smilax*, and *Ruscus* from the Monocotyledonae subdivision. They are glycosides with a triterpenic aglycone structure in dicots or a steroidal aglycone in monocots. They have cardioprotective, anti-inflammatory, anti-viral, and immunoregulatory effects. *Araliaceae, Leguminosae, Polygalaceae*, and *Campanulaceae* families are important sources for saponins. Saponins show an anti-cancer property with different pathways such as proliferation, metastasis, angiogenesis, and autophagy regulation.[22] Studies have shown that ginsenoside, Rg3, obtained from ginseng, which is among plant-derived saponins, suppresses the invasive and metastatic capacity of lung and ovarian cancer cells, and induces apoptosis of melanoma cells.[23–25]

In gastrointestinal cancer, saponins regulate many cancer signaling pathways, affect the immune system, and interact with various transcription molecules against inflammation.[26] There is another study to evaluate the anticancer activity of total saponins from the *Camellia oleifera* Abel. in hepatoma-22 tumor-bearing mice. Saponins induced cancer cell apoptosis through the effect of the antiapoptotic factors, which upregulate the protein expression of Bax and downregulate the protein expression of Bcl-2.[27] α-hederin is triterpenoid saponin in *Nigella* species. In a study demonstrate the pro-apoptotic effects of α-hederin on breast cancer cells through the apoptotic pathway. For MTT assay, breast cancer cell lines were treated with α-hederin (0.08, 0.4, 2, and 10 μg/mL) for 12, 24, and 48 h. α-hederin induces caspase-3 and caspase-9 activation and promote apoptosis of MCF-7 and MDA-MB-231 cells.[28]

There are many researches on the activity of saponins in breast cancer. It has been suggested that sa-

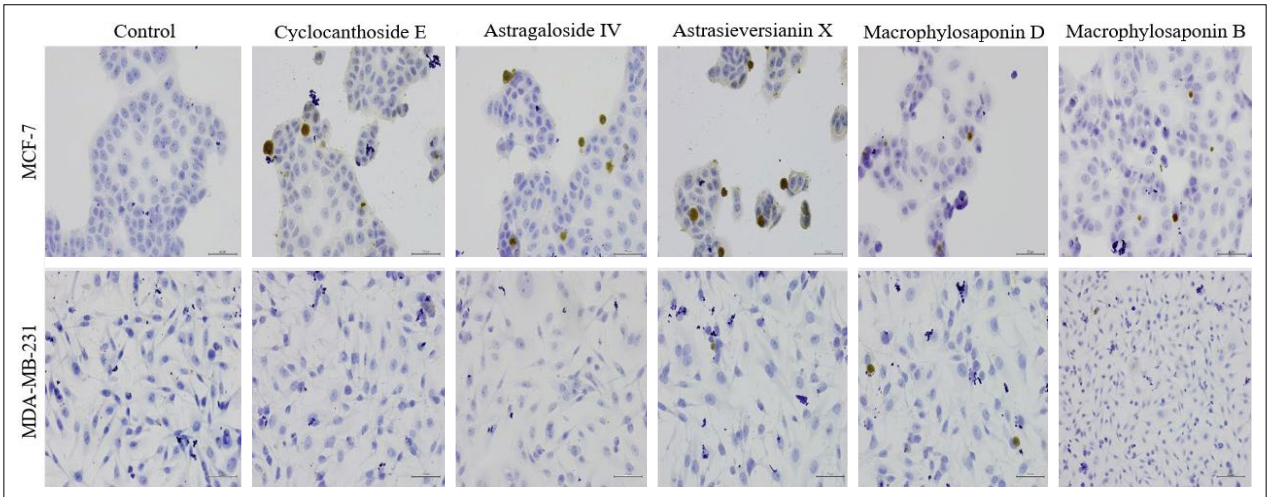

Fig. 4. TUNEL stained breast cancer cell lines are seen in x20 objective magnification.

ponins have anticancer, cytotoxic, proapoptotic, and anti-invasive effects. It has been proven that ginseng saponins can create a valid therapeutic effect for breast cancer treatment by demonstrating its anti-metastatic effect against 4T1 cells metastatic breast cancer cell.[29]

A study demonstrated *Astragalus* species has an anticancer effect with its immunostimulating activity [30]. Astragaloside IV, Astrasieversianin X, Cyclocanthoside E, and Macrophylla Saponin B and D are saponins isolated from *Astragalus* species. Saponins obtained from *Astragalus* species have anti-inflammatory, antioxidative, and anti-cancer activities.[5]

Astragalus polysaccharides with chemotherapy can prevent tumor development, decrease the toxicity of chemotherapy, increase immunity and heal the quality of cancer patient life.[31] In another study, breast cancer invasion and migration were inhibited with *Astragalus* polysaccharides by affecting the epithelial-mesenchymal transition pathway.[32]

Apatinip and *Astragalus* polysaccharides, which are used in the gastric cancer therapy, inhibit the increase of cancer cells depending on the dose and cause an increase in apoptosis.[33] In another tumor xenograft *in vivo* study, it was observed that *Astragalus* saponins combined with adjuvant chemotherapeutics could attenuate cancer development through various action pathways such as regulation of angiogenesis.[34]

In our study, possible cytotoxic and apoptotic activities of saponins obtained from *Astragalus* species on breast cancer cell lines were revealed in the light of research investigating the anticancer properties of saponins. This is the first study investigating the antitumor properties of different saponin extracts from *Astragalus* species in breast cancer. The result of the research, low dose saponin extracts reduce the viability of cell lines, more significantly in MCF-7 cells, increased apoptosis in MCF-7 cell line. Cell viability as evaluated with the CCK-8 test, which is one of the cytotoxicity methods, and the anticancer effects of saponins were examined. Significant cytotoxic effects were demonstrated in low doses at 48 and 72 h. With these conclusions, it has been proven that *Astragalus* saponins inhibit breast cancer cells proliferation depending on a dose and time. In one report, the antitumor activity of total saponins of *Paris forrestii* on human prostate cancer cell lines (PC3) was evaluated. Cells were treated with 0, 2, 4, 6, 8, and 10 μM saponins for 6, 24, and 48 h. As a result, 1 μM and 2 μM of saponins significantly suppressed the invasion and migration of PC3 cells. Saponins of *P. forrestii* can induce apoptosis of human prostate cancer cell lines at very low doses.[35]

Apoptosis essential in the assessment of anticancer properties of plant extracts. Apoptosis is the elimination of unwanted and unrepaired cells. Apoptosis is among the aims of cancer treatment methods such as chemotherapy. The level between cell proliferation and cell death is disrupted in cancer. Fact that natural compounds that stimulate cell death can be used as drugs cause an increase in studies on this subject. There are studies showing the cytotoxic effects of saponins through apoptosis to prevent cancer formation.[36] Apoptotic activities of cycloartane-type saponins used in this study were observed in breast cancer cell lines by TUNEL staining method and statistically significant differences were considered in MCF-7 cell lines.

These results showed that saponins obtained from *Astragalus* have important antiproliferative and anti-apoptotic effects in the MCF-7 cell line, the data will provide a source for further studies on the subject.

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