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Original Article

The effects of *Astragalus* polysaccharides, tragacanthin, and bassorin on methotrexate-resistant acute lymphoblastic leukemia

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Abstract

Background and purpose: One strategy to overcome methotrexate (MTX) resistance in acute lymphoblastic leukemia is suppressing *MDR1* expression. It has been proved *Astragalus* polysaccharides (APS) exert their anticancer effect by reversing drug resistance. Due to the structural similarity of tragacanthin and bassorin with APS, we aimed to investigate the effects of the aforementioned polysaccharides on the expression of the *MDR1* gene in the MTX-treated CCRF-CEM cells.
Experimental approach: Cytotoxicity of APS, bassorin, and tragacanthin on CCRF-CEM, CCRF-CEM/MTX (cells treated with MTX at IC_{50}), and CCRF-CEM/R cells (CCRF-CEM cells resistant to MTX) was evaluated by MTT assay. The effect of all three compounds on *MDR1* expression was evaluated using RT-PCR.
Findings/Results: All the concentrations of tragacanthin, bassorin, and APS (except at 0.8-100 µg/mL in CCRF-CEM) decreased the viability of all the cells compared to the negative control group; and against the positive control (MTX-treated cells), only bassorin at 20-100 µg/mL in CCRF-CEM/R and tragacanthin at 50 and 100 µg/mL in CCRF-CEM/MTX and at 2-100 µg/mL in CCRF-CEM/R decreased cell viability. Tragacanthin diminished *MDR1* expression in CCRF-CEM/MTX and CCRF-CEM/R cells, which MTX had already induced.
Conclusion and implication: According to the results of this study, tragacanthin was a potent cytotoxic agent against CCRF-CEM cells and enhanced the chemosensitivity of CCRF-CEM/MTX and CCRF-CEM/R cells to MTX by down-regulation of MDR1 gene expression. Therefore, it could be a promising compound against cancer. Other possible mechanisms of action of tragacanthin should be evaluated and further *in vitro* and *in vivo* investigations are required.

Keywords: ALL; APS; Bassorin; MDR1 gene; Tragacanthin.

INTRODUCTION

Resistance to chemotherapeutic drugs which limits chemotherapy treatment efficacy is a major concern in the treatment of T-cell acute lymphoblastic leukemia (ALL) which is the most prevalent malignancy among children (1,2). Although based on the studies carried out so far, a 90% 5-year overall survival rate has been reached; with the conventional approach, not only chemotherapy tolerance is still a hindrance that prevents applying of high doses of chemotherapy drugs, but disease recurrence has been regarded as a demanding issue concerning the cancer treatment (3).

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Cellular drug resistance can affect clinical outcomes after chemotherapy. Drug transporter is one of the various mechanisms responsible for drug resistance (4,5). ATP-binding cassette (ABC) transporters are transmembrane proteins that mediate resistance and lead to ineffective chemotherapy by efflux chemotherapeutic drugs and cytotoxic compounds from cancer cells, therefore leading to a decrease in cellular drug accumulation and clinical treatment failure (4,6). ABC transporters' superfamily comprises more than 40 different proteins such as permeability glycoprotein (P-gp), multidrug resistance-associated proteins (MRP), breast cancer resistance protein (BCRP), *etc.* (4,6). Although these transporters have been assessed in several studies, there is controversy about their role in the development of drug resistance in pediatric ALL (5). The multidrug resistance gene (*MDR1*/*ABCB1*) is responsible for encoding P-gp in humans. P-gp, which is overexpressed in neoplastic cells, effluxes toxic compounds and drugs from cancer cells (7).

Methotrexate (MTX) is one of the most essential components of chemotherapeutic drugs applied for the treatment of ALL (1,8,9). It has been proved that members of the MDR efflux transporters such as MDR1 (P-GP), MRP1-5 (ABC1-5), and BCRP (ABCG2) are involved in MTX efflux. Over-expression of these transporters can lead to MTX resistance (8-12).

One strategy to overcome the difficulties associated with P-gp is using *MDR1* expression suppressers which can down-regulate the *MDR1* gene RNA level (13).

Up to date, several herbal components such as glycosides and flavonoids have been identified that can act as P-gp inhibitors (12,13). *Astragalus* has many species which are known to have numerous compounds with pharmacological activity. It has been proved that the dried root of *A. membranaceus* has anticancer effects by increasing immune response, preventing angiogenesis, inducing apoptosis, and reversing MDR (14,15). Also, it can improve chemotherapy outcomes by increasing the sensitivity to chemotherapeutics and reducing their side effects (16). Active pharmacological compounds in the dried root of *A. membranaceus* include polysaccharides, flavonoids, astragalosides, amino acids, and trace elements. The main ingredients of *A. membranaceus* are *Astragalus* polysaccharides (APS). Recently they have been widely investigated, in terms of their wide broad properties and biological activities such as immunopotentiation, reduction of the adverse effects of chemotherapy, hepatoprotection, anti-inflammation, antioxidant, and antitumor. However, the anticancer mechanisms of APS and its effects on reversing drug resistance are not completely clear (17,18). Gum tragacanth (GT) is a dried, natural, polysaccharide from stems and branches of various species of *Astragalus* like *A. gossypinus* which grows in some regions of Iran. It has been reported that GT from different species of *Astragalus* consists of 2 fractions. One fraction, termed 'tragacanthic acid' or bassorin is water insoluble. It contains L-fucose, D-xylose, D-galacturonic acid, D-galactose, and L-rhamnose. Another fraction termed tragacanthin is water soluble. It contains sugars such as L-arabinose, L-fucose, D-mannose, D-glucose, D-galactose, and D-xylose (19,20).

To the best of our knowledge, there is no comprehensive study on the cytotoxic activity and anticancer effect of two distinct parts of GT. Also, it has been shown that APS can decrease *ABCB1* expression. Some studies showed that *ABCB1* can decrease the efficacy of anticancer drugs by induction of their efflux (14). Based on these findings and the structural similarity of two parts of GT with APS (17,21-25), we sought to determine the cytotoxic effect of *A. gossypinus* gum and APS on MTX-sensitive and -resistant CCRF-CEM cells and their effect on the expression of *MDR1*.

MATERIALS AND METHODS

Materials

MTX was obtained as a 25 mg/mL pharmaceutical solution (MYLAN, France) and stored at 4 °C. APS (20000-60000 mol/L) was purchased from Shanxi Undersun Biomedtech Co. Ltd (China). GT was purchased from markets in Isfahan province, I.R. Iran. Taxonomic identification of the specimen was done by the Research Center for Agriculture

and Natural Resources of Isfahan. Other compounds used were 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; ATOCEL, Austria), penicillin/streptomycin (Gibco, USA), RPMI1640, fetal bovine serum (FBS; Biosera, UK), dimethyl sulfoxide (DMSO; Samchun, Korea), Trizol (Innotrain, Germany), cDNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit, Thermo Scientific, UK), SYBR Green (Maxima SYBR Green/ROX qPCR Master Mix (2X), Thermo Scientific, UK).

Cell line and sub-lines

CCRF-CEM cell line was purchased from the cell bank of Pasture Institute of Iran, Tehran, I.R. Iran. CCRF-CEM/MTX subline is the CCRF-CEM cells exposed to MTX 0.1 µM (MTX IC_{50}) for 48 h. It should be noted that the IC_{50} of MTX was determined using different concentrations of it on CCRF-CEM cells through MTT assay (Fig. 1).

MTX resistant subline (CCRF-CEM/R) was obtained by exposing CCRF-CEM cells to continuously increasing concentrations of MTX starting with 5 nM. CCRF-CEM cells were maintained in each concentration of MTX for 2-3 passages until the cells reached their regular growth rate. Methotrexate concentration was increased by 4 folds after every full growth recovery. The entire procedure took approximately 5 months yielding a resistant subline able to grow at 0.6 µM MTX. This protocol was carried out based on previous research (26,27).

CCRF-CEM and CCRF-CEM/MTX were maintained in RPMI-1640 medium supplemented with 10% FBS. CCRF-CEM/R cells were supplemented with MTX 0.6 µM in addition to mentioned media. Cells were passaged twice weekly. All experiments were conducted with cells in the logarithmic growth phase. All of the cells were incubated under standard culture conditions (5% CO_2 and 37 °C).

Quantitative and qualitative control of GT

Identification, measurement of flow time, and total ash of GT were carried out as described in British Pharmacopoeia (28).

Purification of GT

The dried ribbon-like of GT was powdered using a high-speed mechanical blender and then passed through a sieve to obtain uniform powdered samples with a mesh size between 200 to 500 mm. The dried raw powdered tragacanth (5 g) was soaked with ethanol 96% (15 mL). Pure deionized water (1000 mL) was added and the mixture was kept on a magnetic stirrer at room temperature and gently stirred overnight. The obtained solution was completely homogenized using a blender. Then, the freshly prepared solution of alpha-amylase was added to the obtained gum dispersions; after 60 min iodine test was done (29).

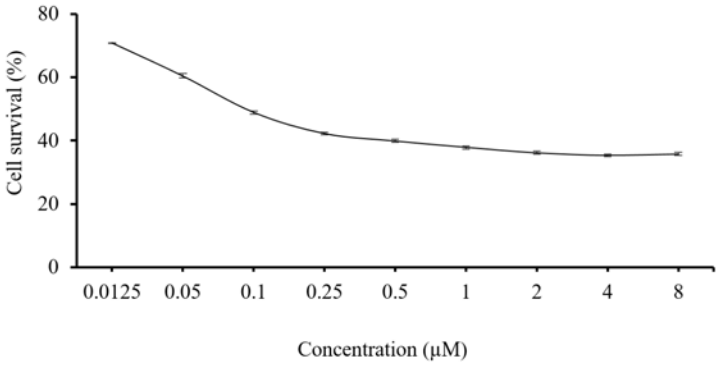

Fig. 1. Determination of the IC_{50} of methotrexate in CCRF-CEM. Data are presented as mean ± SEM, n = 3.

Separation of the soluble and insoluble fractions of GT

To separate the soluble and insoluble fractions, obtained gum dispersions were centrifuged at 16000 rpm for 15 min. The lower gelatinous insoluble part was washed with ethanol and diethyl ether and then freeze-dried. Four volumes of ethanol were added to the supernatant (soluble fraction) and then the gum precipitate was left to rest at 4 °C overnight to ensure complete precipitation. This component was also lyophilized after washing with ethanol and diethyl ether. After lyophilization, both parts were sealed in plastic zip bags and kept in a desiccator for future use. It merits a mention to prepare the desired concentrations of insoluble part of GT, bassorin, distilled water with DMSO (0.5% v/v) was used.

MTT assay

Cell growth and viability were determined by MTT assay. Briefly, 180 µL of cell suspension (1×10^5 cells per mL of media) was seeded in 96-well microplates. After 24 h incubation, the cells were divided into several groups; the sensitive cell line into three separate groups received 20 µL of different final concentrations of APS (0.8, 4, 20, 100, and 500 µg/mL), tragacanthin (2, 5, 10, 20, 50, and 100 µg/mL), or bassorin (2, 5, 10, 20, 50, and 100 µg/mL), CCRF-CEM/MTX cells concomitantly received 10 µL of APS, tragacanthin, and bassorin in combination with 10 µL MTX (at IC_{50} concentration, 0.1 µM). For the resistant cell line (CCRF-CEM/R) same procedure was repeated. To keep consistency concerning the concentration of the treatments in all three cell lines, in CCRF-CEM/MTX and CCRF-CEM/R cells, the concentrations of APS, tragacanthin, and bassorin were doubled.

Then, all plates were incubated for another 48 h. Regarding sensitive sublines, CCF-CEM and CCRF-CEM/MTX, CCRF-CEM cells, and concerning resistant subline, CCRF-CEM/R cells without any treatment were used as negative controls. And where cells were treated with bassorin, DMSO (0.5% v/v) was also added to the wells. CCRF-CEM and CCERF-CEM/R cells treated with MTX at 0.1 and 0.6 µM, respectively, were considered positive controls. Wells containing only media were used as blank. To evaluate cell survival, 20 µL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 3 h. Then, 150 µL of the old medium was gently discarded and replaced by DMSO and pipetted to dissolve any formed formazan crystals.

Absorbance was then determined at 540 nm by an enzyme-linked immunosorbent assay (ELISA) plate reader. Each extract concentration was assayed in 4 wells and repeated 3 times.

RNA extraction and cDNA synthesis

CCRF-CEM, CCRF-CEM/R cells (2×10^6 cells/mL) were seeded in 6 wells plates. After 24 h incubation, 3 different treatments were done:

CCRF-CEM cells were treated with APS (500 µg/mL), tragacanthin (2 and 10 mg/L), or bassorin (20 and100 µg/mL). CCRF-CEM cells were used as control.

CCRF-CEM/MTX cells treated with APS (500 µg/mL), tragacanthin (2 and 10 µg/mL), or bassorin (20 and 100 µg/mL). CCRF-CEM and CCRF-CEM/MTX cells were used as negative and positive controls, respectively.

CCRF-CEM/R, treated with APS (500 µg/mL), tragacanthin (2 and 10 µg/mL), or bassorin (20 and 100 µg/mL). CCRF-CEM cells and CCRF-CEM/R were used as negative and positive controls, respectively.

After incubation for 48 h isolated cells were washed twice with PBS followed by total cellular RNA extracted from a minimum of 1×10^6 cells using the Trizol method according to the manufacturer's instruction. The extracted RNA was quantified spectrophotometrically using a biophotometer (Eppendorf, USA) and reverse transcription was done from 2 µg of total RNA according to the protocol provided by the cDNA synthesis kit using a random hexamer primer, RevertAidTM M-MuLV reverse transcriptase and thermocycler (TaKaRa) in a specific temperature plan (sequentially: 5 min at 25 °C, 60 min at 42 °C, and 5 min at 70 °C). The concentration and quality of the cDNA were estimated using a NanoDrop (Epoch, USA). The obtained cDNA was preserved at -70 °C for future steps.

Table 1. The primers' sequences of the endogenous control (GAPDH) and MDR1 genes.

Gene	Primer sequences (5′ →3′)	Primer length (bp)	Product size (bp)
GAPDH	F: GCCCCAGCAAGAGCACAAGAGGAAGA	26	106
	R: CATGGCAACTGTGAGGAGGGGAGATT	26	
MDR1	F: GAGGCCGCTGTTCGTTTCCTTTAGGTC	27	109
	R: AGATTCATTCCGACCTCGCGCTCCT	25	

Real-time polymerase chain reaction

To determine whether APS, bassorin, and tragacanthin influence MTX resistance in CCRF-CEM cells, the relative mRNA expression levels of *MDR1* in the CCRF-CEM cell lines, sensitive and resistant to MTX, were assessed by quantitative real-time polymerase chain reaction (RT-PCR) with SYBR Green (Maxima SYBR Green/ROX qPCR Master Mix (2X), UK) in a Step One Plus RT-PCR machine (Applied biosystem, Faster city, CA, USA). The total volume of the RT-PCR reaction, 10 µL, contained 1.6 µL synthesized cDNA (0.1 µg/µL), 0.4 µL of 10 µM solutions of each forward and reverse primers, 5 µL of SYBR green, and 2.6 µL of ddH_2O. Primer sequences are summarized in Table 1. These primers were adopted from previous studies (5). The specificity of the primers was verified by Blast analysis at NCBI.

The $2^{-\Delta\Delta Ct}$ method was used to determine the relative expression ratio between the target gene (MDR1) and the housekeeping gene used as an internal control (glyceraldehyde-3-phosphate dehydrogenase, GAPDH). Data were analyzed with sequence detector software (Step One v2.3, Applied Biosystems, USA).

Statistical analysis

Statistical analysis for the data of cell cytotoxicity and *MDR1* gene expression assays was performed using GraphPad Prism 6 software. The differences in variables between the groups were analyzed by one-way ANOVA followed by Tukey's multiple comparison post-test. *P*-values < 0.05 were considered significant.

RESULTS

Gum tragacanth characterization

Observation of an intense yellow color after adding barium hydroxide confirmed the presence of GT. Flow time was obtained at 70 ± 3 s, and total ash was 1.6 ± 0.1%.

Evaluation of the cytotoxic effect of APS, tragacanthin, and bassorin

Concentration-dependent growth inhibition was induced by tragacanthin and bassorin on the leukemic cells (CCRF-CEM, CCRF-CEM/MTX, and CCRF-CEM/R) when applied alone or in combination with MTX at 0.1 and 0.6 µM, respectively. APS, alone, at 100 and 500 µg/mL reduced CCRF-CEM cell viability, however, it couldn't induce any significant changes in CCRF-CEM/MTX and CCRF-CEM/R cells viability compared with the positive control groups. In all the cells, tragacanthin had a more cytotoxic effect relative to others (Figs. 2-4).

The effect of APS, tragacanthin, and bassorin on MDR1 expression

Comparing the relative mRNA expression of the *MDR1* gene showed that the levels of *MDR1* mRNA expression in both sensitive and resistant cells (CCRF-CEM/MTX and CCRF-CEM/R cells), when treated with MTX, were significantly higher than that of the sensitive cells without MTX treatment (Tables 2-4). As expected, there was no significant difference in the levels of relative *MDR1* mRNA expression between resistant cells with and without MTX exposure. APS decreased *MDR1* mRNA expression only in CCRF-CEM/MTX and CCRF-CEM/R cells which were insignificant compared to the positive control group. Tragacanthin (2 and 10 µg/mL) decreased *MDR1* mRNA expression, concentration-dependently, in CCRF-CEM/MTX and CCRF-CEM/R sublines compared to positive control. Bassorin (20 and 100 µg/mL) decreased *MDR1* mRNA expression only in CCRF-CEM/MTX subline in comparison with cells exposed to MTX and in resistant cells it led to a considerable gene expression increase at 100 µg/mL.

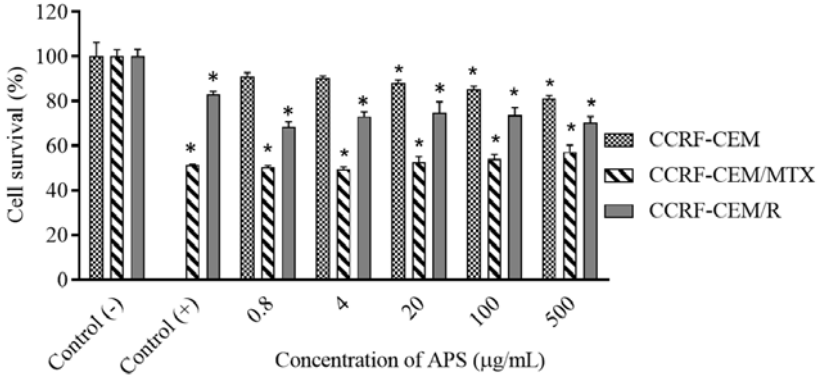

Fig. 2. The effects of APS on the viability of CCRF-CEM cells. CCRF-CEM cells were treated with APS alone, CCRF-CEM/MTX cells received APS in combination with MTX at 0.1 μM, and CCRF-CEM/R cells were treated with APS in combination with MTX at 0.6 μM. Results are shown as mean ± SEM of 3 experiments. *$P < 0.05$ Indicates significant differences compared to the respective negative control group (cells that did not receive any treatments). CCRF-CEM and CCERF-CEM/R cells treated with MTX at 0.1 and 0.6 μM, respectively were considered positive controls. APS, *Astragalus* polysaccharides; MTX, methotrexate.

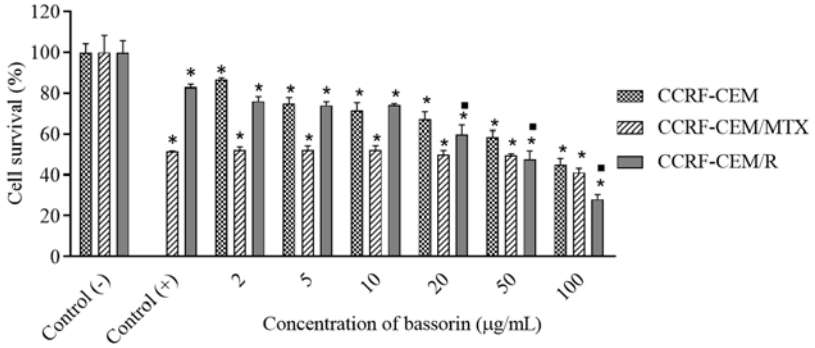

Fig. 3. The effects of bassorin on the viability of CCRF-CEM cells. CCRF-CEM cells were treated with bassorin alone, CCRF-CEM/MTX cells received bassorin in combination with MTX at 0.1, and CCRF-CEM/R cells were treated with bassorin in combination with MTX at 0.6 μM. Results are shown as mean ± SEM, n = 3. *$P < 0.05$ Indicates significant differences compared to negative control groups (cells that did not receive any treatments) and ▪$P < 0.05$ *vs* positive control groups (CCRF-CEM and CCERF-CEM/R cells treated with MTX at 0.1 and 0.6 μM, respectively). APS, *Astragalus* polysaccharides; MTX, methotrexate.

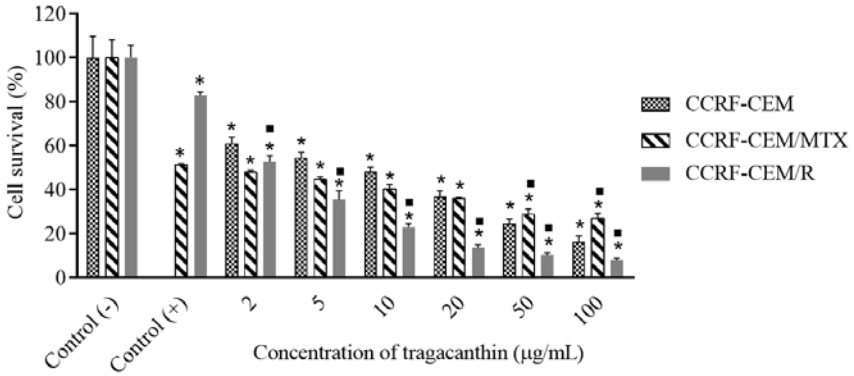

Fig. 4. The effects of tragacanthin on the viability of CCRF-CEM cells. CCRF-CEM cells were treated with tragacanthin alone, CCRF-CEM/MTX cells received tragacanthin in combination with MTX at 0.1, and CCRF-CEM/R cells were treated with tragacanthin in combination with MTX at 0.6 μM. Results are shown as mean ± SEM, n = 3. *$P < 0.05$ Indicates significant differences compared to negative control groups (cells that did not receive any treatments) and ▪ $P < 0.05$ *vs* positive control groups (CCRF-CEM and CCERF-CEM/R cells treated with MTX at 0.1 and 0.6 μM, respectively). APS, *Astragalus* polysaccharides; MTX, methotrexate.

Table 2. *MDR1* mRNA expression in CCRF-CEM cells in 48 h exposure to APS, tragacanthin, and bassorin. Data are expressed as mean ± SEM. **P* < 0.05 Shows significant differences in comparison with the control group (CCRF-CEM cells).

Groups	*MDR1* expression
APS (500 µg/mL)	2.32 ± 0.4
Tragacanthin (2 µg/mL)	1.18 ± 0.2
Tragacanthin (10 µg/mL)	0.42 ± 0.08*
Bassorin (20 µg/mL)	2.20 ± 0.2
Bassorin (100 µg/mL)	3.89 ± 0.4*
Control	1.00

APS, *Astragalus* polysaccharides; MDR, multidrug resistance gene.

Table 3. *MDR1* mRNA expression in CCRF-CEM/MTX cells in 48 h exposure to APS, tragacanthin, and bassorin. **P* < 0.05 Indicates significant differences compared to the control group (CCRF-CEM cells without any treatment), $^{\#}P < 0.05$ *vs* CCRF-CEM/MTX cells.

Groups	*MDR1* expression
APS (500 µg/mL)	4.99 ± 0.3*
Tragacanthin (2 µg/mL)	1.63 ± 0.1$^{\#}$
Tragacanthin (10 µg/mL)	0.63 ± 0.05$^{\#}$
Bassorin (20 µg/mL)	1.48 ± 0.1$^{\#}$
Bassorin (100 µg/mL)	2.73 ± 0.2*,$^{\#}$
CCRF-CEM/MTX	6.41 ± 0.5*
Control	1.00

CCRF-CEM/MTX, CCRF-CEM cells treated with methotrexate at IC_{50} (0.1 µM); APS, *Astragalus* polysaccharides; MDR, multidrug resistance gene.

Table 4. MDR1 mRNA expression in CCRF-CEM/R cells in 48 h exposure to APS, tragacanthin, and bassorin. **P* < 0.05 Indicates significant differences in comparison with the control group (CCRF-CEM cells); and also $^{\#}P < 0.05$ *vs* CCRF-CEM/R cells.

Groups	*MDR1* expression
APS (500 µg/mL)	2.34 ± 0.2
Tragacanthin (2 µg/mL)	1.06 ± 0.1$^{\#}$
Tragacanthin (10 µg/mL)	0.81 ± 0.1$^{\#}$
Bassorin (20 µg/mL)	1.88 ± 0.2
Bassorin (100 µg/mL)	5.74 ± 0.5*,$^{\#}$
CCRF-CEM/R	3.03 ± 0.2*
CCRF-CEM/R + MTX (0.6 µM)	3.04 ± 0.2*
Control	1.00

CCRF-CEM/R cells, CCRF-CEM cells resistant to methotrexate at 0.6 µM; APS, *Astragalus* polysaccharides; MDR, multidrug resistance gene

DISCUSSION

MTX is a common therapy for ALL patients. Although the high dose of MTX can significantly increase cure rates and improve patients' prognosis (30), increased MTX plasma concentration leads to numerous adverse effects (10). To reduce the side effects of chemotherapy several studies have been conducted on the effects of natural polysaccharides in cancer treatment (31,32). Anticancer and chemosensitizing effects of APS have recently attracted attention to overcome some difficulties during the treatment process (33-35). It has been shown that APS in combination with therapeutic drugs can decrease the adverse effects of chemotherapy, and elevate immune function *via* inhibiting tumor development (36-38). The results of the present study revealed that applying APS alone significantly decreased the viability of the cells compared to the negative control groups, however, its impact on decreasing the viability of both sensitive and resistant cells was not considerable compared to the positive controls, which means its combination with MTX couldn't cause any significant alteration in cytotoxicity induced by MTX. In this regard, our finding was not consistent with the findings of the research claimed that APS could improve chemotherapy outcomes by increasing the sensitivity to chemotherapeutics and reducing their side effects (16). This discrepancy might happen owing to several reasons, type of cell line for instance. We obtained interesting and promising results regarding the cell toxicity of bassorin and tragacanthin.

To the best of our knowledge, the anticancer effects of GT have not yet been investigated. Bassorin and tragacanthin showed a remarkable and concentration-dependently cytotoxic effect on sensitive and resistant CCRF-CEM cells. Their cytotoxic effect has been expected, due to their polysaccharide structures, as findings by Shu *et al*. showed that polysaccharides from *S. chinensis* suppressed *in vivo* proliferation of H22 cells without toxic effects on tumor-bearing mice (31). It has been shown that a water-soluble polysaccharide isolated from the

leaves of *Ampelopsis megalophylla* inhibited the growth of transplantable Sarcoma 180 tumor in mice and increased the spleen index and body weight (32).

The drug resistance phenomenon in leukemic cells is one of the reasons for treatment failure (39). Overexpression of MDR1/P-gp and its function is associated with failure of chemotherapy in ALL patients (40,41). It has been reported resistance to MTX in CCRF-CEM cells is related to the overexpression of MDR1, which in turn leads to overexpression of P-gp resulting in more effluxing of MTX which is a substrate of P-gp (42,43). But there is a controversy about the role of MDR1 in clinical outcome failure in patients with ALL (4,44,45). Kourti *et al*. reported that high expression of the MDR1 gene in ALL patients led to significantly worse prognoses (46). In contrast, some researchers have reported that MDR1/P-gp is unlikely to be involved in drug resistance in pediatric ALL and also their findings did not support the transportation of MTX by P-gp (47-49).

According to the results of the current study obtained by RT-PCR the level of MDR1 expression in the cells treated with MTX, CCRF-CEM/R and CCRF-CEM/MTX cells, were 3 and 6.4 folds more than that of CCRF-CEM cells. In line with our results, Norris *et al.* showed that MDR1/P-gp can contribute to MTX resistance in the humane leukemic CCRF-CEM cell line (50). Also, it has been found that ABCB1 polymorphism may have an important role in the development of ALL (51). In our study, APS did not affect MDR1 expression in CCRF-CEM cells by itself when applied alone, and also its simultaneous application with MTX in both MTX-resistant and sensitive CCRF-CEM cells couldn't decrease the gene expression compared to the positive control. Therefore, based on the obtained results by MTT assay and RT-PCR, it could be concluded that APS was not able to amplify the cytotoxicity induced by MTX in both resistant and sensitive CCRF-CEM cells. Interestingly, tragacanthin and bassorin followed two opposite trends in order to regulate MDR1 expression. When tragacanthin was applied alone or in combination with MTX (in both sensitive and resistant cells) led to gene downregulation, however, bassorin increased the expression of desired gene concentration-dependently. So, it could be concluded that bassorin might escalate the cell toxicity induced by MTX through other mechanisms like facilitated cell apoptosis which could be further evaluated using some experimental methods such as flow cytometry. Therefore, two parts of GT could be separately used either for increasing or decreasing MDR1 gene expression.

This investigation has proposed a new insight into the effects of both parts of GT on MDR1 gene expression. Tragacanthin could be promising to overcome the MTX resistance in ALL patients by enhancing the chemosensitivity of MTX through downregulating MDR1 gene expression. Hence, further in vitro and in vivo investigations are required which will give us more information on whether they could be used as co-treatment in ALL patient chemotherapy and also as agents to overcome the resistance to the drugs which are effluxed by P-gp.

CONCLUSION

Tragacanthin could be considered a potent cytotoxic agent affecting both MTX-sensitive and -resistant CCRF-CEM cell viability. Tragacanthin-induced chemosensitivity of CCRF-CEM and CCRF-CEM/R cells to MTX could be related to its effect on the down-regulation of MDR1 mRNA expression.

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Conflict of interest statement

The authors declared no conflicts of interest in this study.

Authors' contribution

B. Samii, A. Jafarian, M. Rabbani, B. Zolfaghari, and S. Rahgozar contributed to the concept and design of the study. All the experiments were carried out by B. Samii.

E. Pouraboutaleb partially assisted in the experimental approach. B. Samii acquired the data. B. Samii, A. Jafarian, M. Rabbani, and B. Zolfaghari interpreted the data. The manuscript was written by B. Samii under the supervision of A. Jafarian, M. Rabbani, B. Zolfaghari, S. Rahgozar. The final draft of the article was approved by all authors.

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