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Cytotoxic and cytogenetic effects of *Convolvulus arvensis* extracts on rhabdomyosarcoma (RD) tumor cell line *in vitro*

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The present study was designed to investigate the cytotoxicity of (aqueous and methanol) crude extracts of the leaves, stems and roots extracts as well as proteoglycan and glycoside fraction I (FI) of *Convolvulus arvensis* against human Rhabdomyosarcoma (RD) tumor cell line *in vitro*. The effect of glycoside FI fraction on mitotic index (MI) of RD cell line was investigated as well. The optical density (OD) of cell growth was measured by Elisa reader at 492 nm using tetrazolium bromide (MTT). Aqueous and methanol leaves extracts and glycoside FI had more cytotoxic effects at 10 mg/ml after 24 h. After 48 h, proteoglycan and glycoside FI at 10 mg/ml revealed very high cytotoxic activity compared with other concentrations. After 72 h, glycoside FI at 10 mg/ml had more cytotoxic inhibition compared with other extracts. Glycoside FI had cytotoxicity concentration 50% (CC 50%) 1.775, 0.870 and 0.706 mg/ml after 24, 48, and 72 h, respectively. The root aqueous extract had less cytotoxic effect after 72 h than other extracts; the CC 50% was 7.437 mg/ml. Cytotoxicity of root aqueous extract was more pronounced at higher concentration of 10 mg/ml. The effect of glycoside FI on MI of RD tumor cell line was concentration dependant.

Key words: Cytotoxicity, cytogenetics, *Convolvulus arvensis*, rhabdomyosarcoma (RD) tumor cell line, mitotic index (MI), tetrazolium bromide (MTT).

INTRODUCTION

Herbal medicine has a vital role in the prevention and treatment of cancer. A great deal of pharmaceutical research output in advanced countries has considerably improved the quality of the herbal medicines used in treatment of cancer (World Health Organization (WHO), 2002). The Convolvulaceae family includes a large number of important plants which have many chemical compounds that are used for treating many diseases (Jacobs and NRCS, 2007).

Convolvulus species are widely distributed all over the world in different localities; some of them have medicinal activity (Abdel-Raheim et al., 2011), such as anticancer, anti-ulcerogenic and antidiarrhoeal. Some species also have a broad activity against some bacteria and fungi (Dhingra and Valecha, 2007). *Convolvulus arvensis* is an example of a medicinal plant with high therapeutic activities. *C. arvensis* is evaluated as a potential new source of antioxidant activity (Mohammed et al., 2011).

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The antioxidant activity of *C. arvensis* extracts is mainly due to phenolic contents such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Awaad and Jaber, 2010; Thakral et al., 2010). *C. arvensis* may be a promising source of anticancer agents (Thane et al., 2000). Different extracts of *C*. *arvensis* affect tumor angiogenesis and immune cell function that stimulate immune cells (Kidd, 2000). Angiogenesis inhibitors derived from natural sources include flavonoids, sulphated carbohydrates and triterpenoids (Paper, 1998). Bind weed extract which contains angiogenesis-suppressing proteoglycan molecules (PGMs) regulates the production of a potent angiogenesis inhibitor, interleukin 12. All the previous researches revealed that no study has been carried out on cytotoxicity and cytogenetic effects of different extracts of *C. arvensis* on rhabdomyosarcoma (RD) tumor cell line. Therefore the present study was conducted to evaluate the cytotoxic effects of eight of extracts of *C. arvensis* and cytogenetic effect of glycoside FI leaves fraction of *C. arvensis* on this cell line.

MATERIALS AND METHODS

Aqueous extracts

An aqueous extract from leaves, stems or roots of *C. arvensis* was prepared according to Harborne (1984). Fifty grams of the dried ground leaves, stems or roots were macerated with 200 ml water over night, at 45°C. After 24 h, the extractive solution was filtered in a double layer of gauze then through filter paper (Whitman No.1). The pooled extract was evaporated to dryness at 45°C under reduced pressure in a rotary evaporator (Orem Sceentific LtdSwiss). The yield of crude extract was weighted and kept at -20°C until use. Half gram of resultant extract was dissolved into 10 ml phosphate buffer saline (PBS). The suspension was filtered and sterilized by using two sterile Millipore filter papers, 0.45 and 0.22 µm and was kept in deep freeze at -20°C as a stock solution until use.

Methanol crude extracts of C. arvensis

Leaves, stems and root methanol extracts were prepared according to Lin et al. (2010). Fifty gram of each part was refluxed with 250 ml of absolute methanol at 60°C for 4 h. The supernatant was separated from the solid residue using Whitman No.1 filter paper. The extraction was repeated twice. The extracts were combined and evaporated at 60°C under reduced pressure. After drying, 0.5 g of the resultant extract was dissolved in 10 ml PBS. The suspension was filtered and sterilized using both 0.45 and 0.22 µm sterile Millipore filter paper and was kept in deep freeze at -20°C as a stock solution until use.

Extraction and fractionation of glycosides from leaves of C. arvensis

Glycosides were separated and fractionated according to Menemen et al. (2002). The resultant extract was filtered and evaporated to dryness under reduced pressure in a rotary evaporator (Orem Scientific Ltd., Swiss). To a fractionation of glycoside, the dried glycoside extract was dissolved in 80% methanol and was run by thin layer chromatography (TLC) using silica gel in n-butanol:acetic acid:water (BAW), 5:1:4 as eluent. The glycoside spots were examined and their position and color reactions recorded and finally the rate of flow (R_f) values were recorded. To obtain a large amount of fractions, we used the protocol of Menemen et al. (2002). Half gram of the FI was dissolved in 10 ml of 1% dimethyl sulfoxide (DMSO) and the suspension filtered and sterilized using 0.45 µ then a 0.22 µ sterile Millipore filter paper kept in deep freeze -20°C as a stock solution to be used later.

Extraction of proteoglycan molecules (PGM) from leaves of C. arvensis

The dried powder of *C. arvensis* leaves was mixed in dry weight (DW) at a concentration of 0.16 g/ml to prepare the proteoglycan as described previously (Meng et al., 2002). The resultant product was lyophilized to produce the extract powder which is referred to as PGM. 0.5 gm of resultant extract was dissolved in 10 ml PBS and the suspension filtered and sterilized using 0.45 µm then a 0.22 µm sterile Millipore filter paper, and finally kept in deep freeze -20°C as a stock solution until use.

Chemical tests

Alkaloids in plant extracts were determined using Wagner's and Dragendorff tests (Harborne, 1984), whereas tannins were determined using ferric chloride and lead acetate solutions (Harborne, 1984). Flavonoids was tested according to AL-Shahaat (1986). Liebermann-Burchard test was used for triterpenoids. Peptides and free amino groups tests were used for peptides, primary or secondary amino groups (Harborne, 1984). Molish reagent test was used for carbohydrate (Hawk et al., 1954). The presence of glycosides was detected according to AL-Shahaat (1986). Saponins were identified according to Harborne (1984). Biuret test was used to detect protein (Saadalla, 1980).

Cell lines

Rhabdomyosarcoma (RD) was provided by Iraqi Center for Cancer and Medical Genetic Research/Baghdad (ICCMGR). Passage number 45 was used and the cells were cultivated in minimum essential medium (MEM) with L-Glutamine and HEPES (Sigma, USA) which was supplemented with 10% of fetal calf serum and penicillin/streptomycin. The following formula was followed to calculate viability of the cell lines using 1% Trypan blue stain (Fine Chemical, Sweden Pharmac): $C = N \times D \times 10^4$, where C is the number of viable cells per milliliter, N is the number of viable cells counted, and D is the dilution factor (D = 10) (Fresheny, 1994).

Effect of aqueous and methanol crude extracts of leaves, stems, root, proteoglycan and glycoside (FI) extracts of *C. arvensis* on growth of RD tumor cell line

About 200 µl of RD cells passage (45) were suspended (55,000 cells/ml) in growth medium. These cells were seeded into each well of a sterile 96-wells micro-titration plate. The plates were sealed with a self-adhesive film, lids were placed on and incubated at 37°C in 5% CO_2 incubator. When the cells are in exponential growth (approximately 70 to 80% confluent monolayer) after 72 h, the medium was removed and serial dilutions of each aqueous, methanol crude extracts of *C. arvensis* in free serum MEM medium were added to the wells. The same was applied for glycoside (FI) and proteoglycan (PGM) separately. The dilutions were 10, 5, 2.5, 1.250, 0.5, 0.313, 0.156 and 0.078 mg/ml, respectively. Three replicates were used for each concentration of each extract. Three wells were used for seeding cells in medium alone, another three

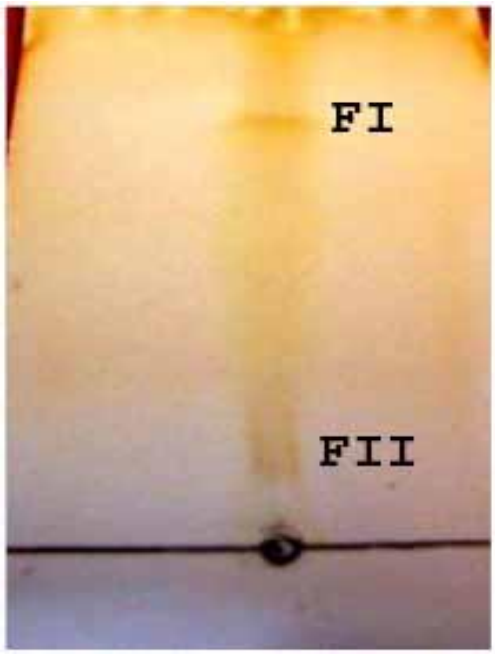

Figure 1. TLC of glycoside fractions developed by H_2SO_4.

wells for seeding cells in medium with PBS and three wells for seeding in medium with DMSO 1% final concentration; we confirmed that this DMSO concentration did not affect the proliferation of RD cell line. The plates were re-incubated at 37°C for the selected exposure times (24, 48, and 72 h). After 24, 48 and 72 h of exposure to each extract, the medium was removed and 28 µl of [3(4, 5-Dimethyl thiazol-2-yl) 2-5-Diphenyl-tetrazolium bromide] MTT (Sigma) were added to the wells. The plates were incubated in CO_2-incubator for 2 h at 37°C. At the end of incubation, the excess dye was removed and 130 µl of DMSO was added to each well to solubilize and extract the dye from the viable cells. Then, the plates were placed on a shaker for 15 min. The optical density (OD) of each well after treatment was read using enzyme linked immunosorbent assay (ELISA) reader at a transmitting wavelength of 492 nm (Betancur-Galvis et al., 2002). The percentage of cytotoxicity was calculated as (A-B) / A × 100, where A means OD of untreated wells and B is the OD of wells with plant extracts (Betancur-Galvis et al., 1999). The cytotoxic concentration 50% (CC 50%) for each extract was calculated from concentration-effect-curves after linear regression analysis (Hayslett and Patrick, 1981).

Cytogenetic effect of glycoside (FI) extract on RD tumor cell line

Cultures of RD tumor cell line (three replicates) were used for treatment with glycoside FI extract since it was a more effective extract to determine the mitotic index (MI). The concentrations of glycoside FI were 0.353, 0.176 and 0.088 mg/ml, which were selected according to the cytotoxicity tests (less than CC 50%). Another set of three culture flasks were used to maintain the media with 1% DMSO only as a negative control. Flasks were incubated at 37°C for 72 h (Modi et al., 1987). Culture in the flask was re-fed with pre-warmed fresh medium 6 h before adding colcemide solution to obtain a final concentration of 1 µg/ml and incubated at 37°C for half an hour. Slides were prepared according to Modi (1987) and stained by using 2% Giemsa stain (Merck, USA) for 2.5 min and rapidly washed with Sorenson's buffer and then left to dry at room temperature. Microscopical examination under 40× objective lens was followed to detect the MI. One thousand cells were examined in each slide to calculate the MI. The MI% was determined as a ratio of the mitotic cells in metaphase to the total cells.

MI% = (No. of dividing cells / No. of dividing cells + No. of non-dividing cells) × 100 (Kleinsmith, 2006).

Statistical analysis

The results were evaluated by the analysis of the variance (ANOVA), P-values at levels ($P \leq 0.01$) were considered to be statistically significant and this calculation was carried out according to statistical package for social science (SPSS, version 19). The least significant difference (LSD) at the level less than 0.05 were used to determine the significant differences between levels of each factor (Steel and Torrie, 1980).

RESULTS

Qualitative chemical analysis of *C. arvensis* extracts

The results of qualitative chemical analysis of *C. arvensis* (aqueous and methanol) crude extracts from (leaves, stems and roots), methanol glycosides (FI and FII) and aqueous proteoglycan from leaves with respect to the yield of extractions % are summarize in Tables 1 and 2.

Thin layer chromatography (TLC) of methanol leaves extract of C. *arvensis*

The results of TLC for methanol leaves extract of *C. arvensis* showed the presence of two brown color spots; the color was developed by H_2SO_4 as shown in Figure 1. These two spots were different in the rate of flow (R_f). FI = 0.8 and FII = 0.187 (Table 3).

Spectrophotometer analysis

The result of fractions of glycosides from leaves of *C. arvensis* showed two peak of Fractions I and II at $\lambda = 450$ nm (Figure 2).

Fourier transforms infra-red (FTIR) spectroscopy analysis of glycoside fractions

Infrared spectrum for glycoside fractions FI and FII extracted from *C. arvensis* leaves are shown in Figures 3 and 4 and the results were illustrated in Table 4.

Melting point

The melting point of Fraction I was at 160°C, but the melting point of fraction II was not detected due to its viscous nature.

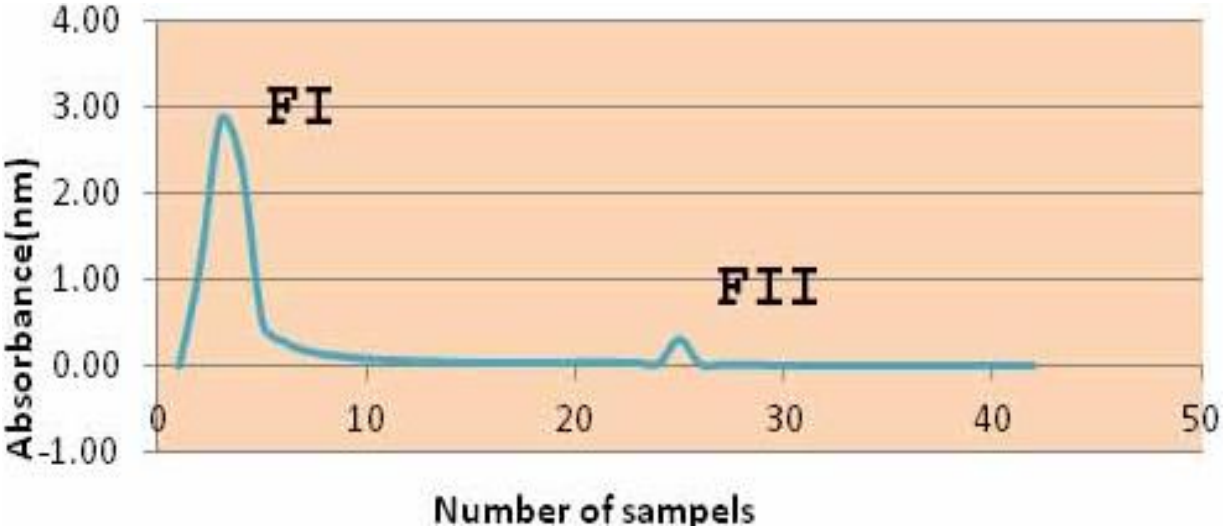

Figure 2. Silica gel chromatography of glycosides extracts from *C. arvensis*.

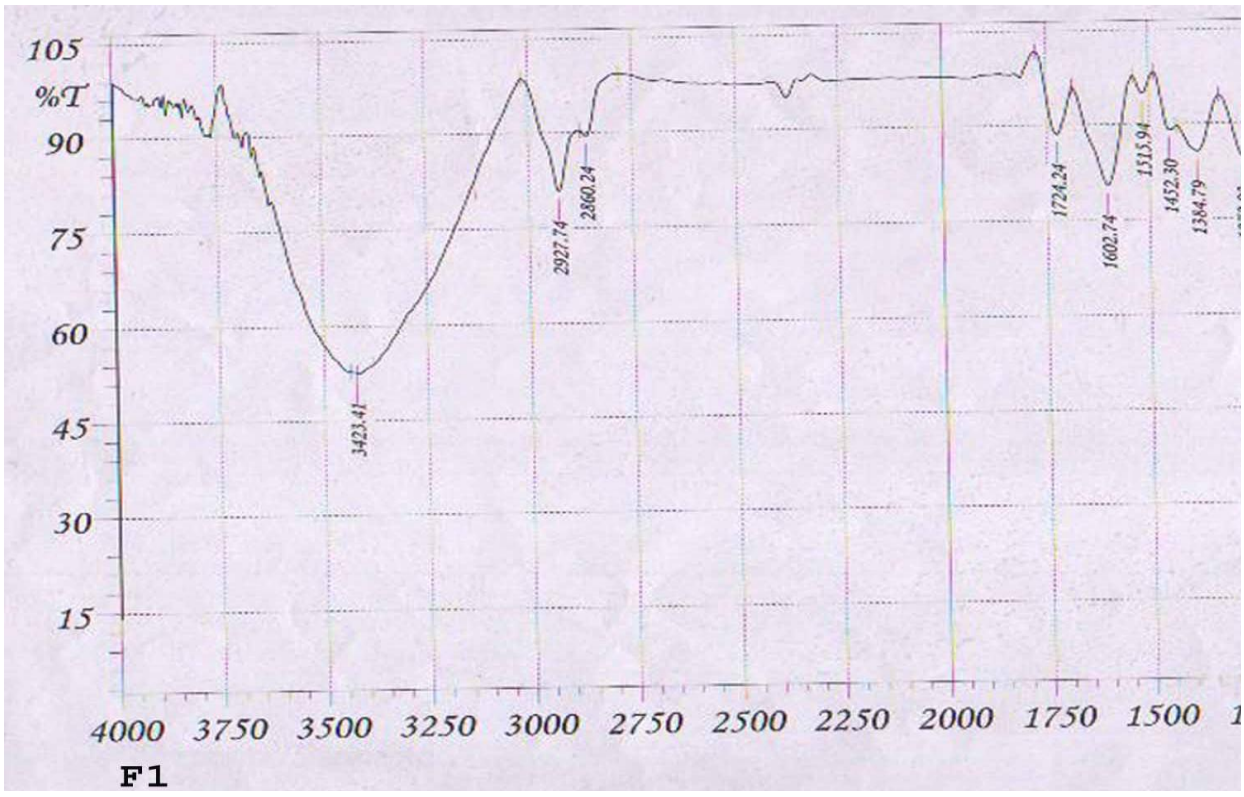

Figure 3. Infrared spectrum for fraction I (FI) of glycoside of *C. arvensis* leaves extract.

Cytotoxic effects of (aqueous, methanol) crude extracts from leaves, stems and roots (glycoside FI and PGM) from leaves of *C. arvensis* on RD tumor cell lines *in vitro*

The results show that the effect of glycoside FI, proteoglycan extracts and all crude extracts(leaves, stems and roots) of *C. arvensis* on proliferation of RD tumor cell line was highly significant ($P \leq 0.001$) in all periods of treatments. The interaction between the effects of extracts and their concentrations was highly significant ($P \leq 0.001$) after all periods of treatments. The foregoing

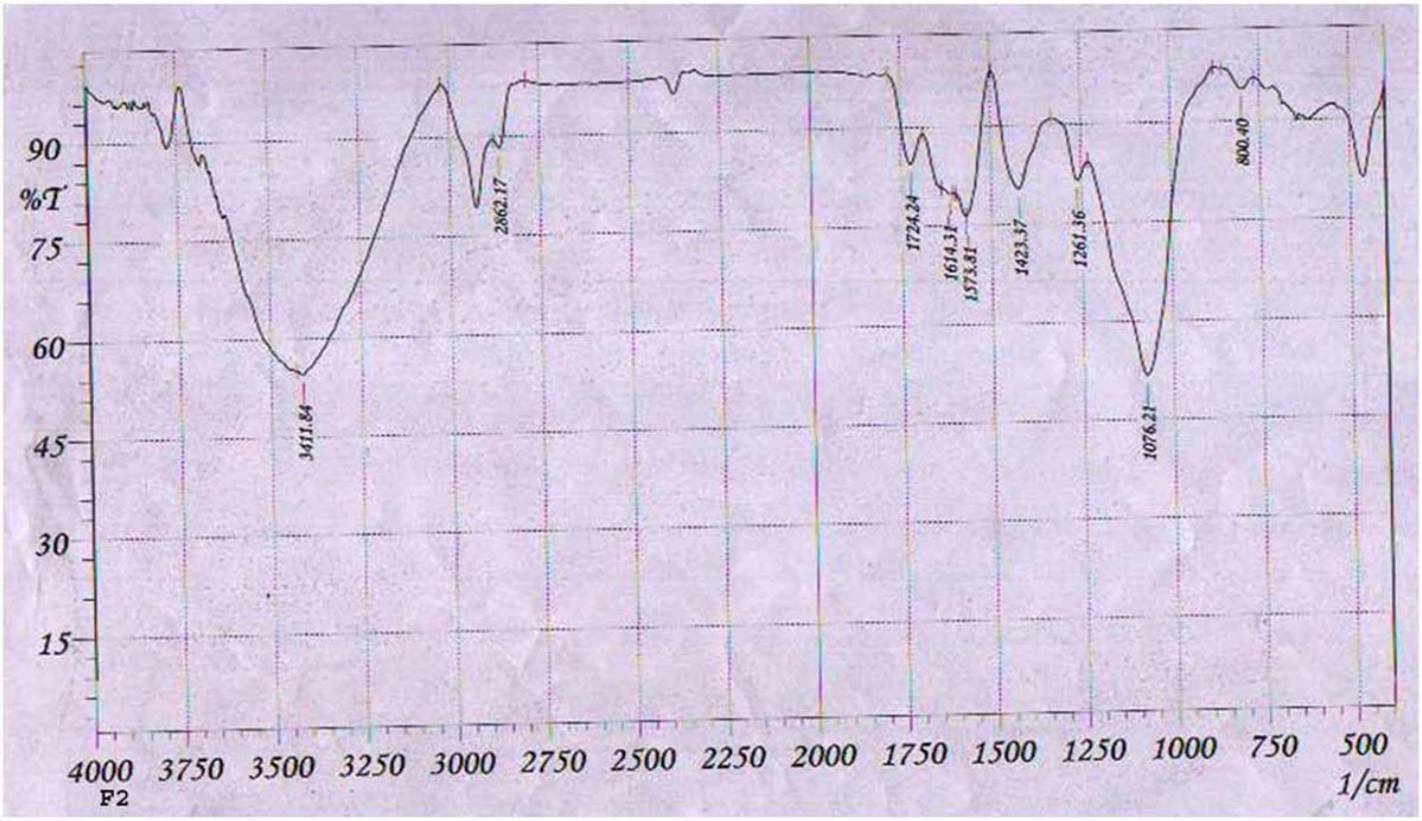

Figure 4. Infrared spectrum for fraction II (FII) of glycoside of *C. arvensis* leaves extract.

Table 1. The yield of extraction % of *C. arvensis* (aqueous and methanol) crude extracts (leaves, stems and roots), methanol glycosides (FI and FII) and aqueous proteoglycan from leaves.

Part of plant	Type of extract		Yield of extraction %
L	Methanol		19.85
L	Aqueous		12.37
L	Proteoglycan		9.28
L	Glycosides	F1	4.22
		F2	0.21
S	Methanol		13.27
S	Aqueous		11.06
R	Methanol		15.38
R	Aqueous		9.80

L: leaf, S: stem R: root.

foregoing results indicate that the toxicity of the leaves stems and roots extracts varied with different types of extracts and concentrations. The concentrations of leaves methanol, leaves aqueous extracts and glycoside FI 10 mg/ml after 24 h show significant effect on growth of RD tumor cell line (Table 5) as compared to the control group which shows complete confluent monolayer of cohesive malignant cells. The effect of each stems aqueous extract, proteoglycan and stem methanol extract at 10 mg/ml was less than that of leaves methanol, leaves aqueous extract and glycoside FI at 24 h. Interaction between concentrations and extracts revealed that leaves methanol, leaves aqueous extracts and glycoside FI extract had the same effect at 10 mg/ml after 24 h. After 48 h, Table 6 shows that the effect of proteoglycan and glycoside FI against the proliferation of RD cells, especially at 10 mg/ml, was more than the effects of all others extracts.

Table 2. Qualitative chemical analysis for aqueous and methanol extracts of leaves, stems, roots, glycosides FI, FII and proteoglycan for leaves of *C. arvensis*.

Compound group	Extract								
	Aqueous			Methanol			Proteoglycan	Glycosides	
	Leaves	Stem	Root	Leaves	Stem	Root		FI	FII
Alkaloids									
Wagner's test	+	+	+	+	+	+	-	-	-
Dragendorf test	+	+	+	+	+	+	-	-	-
Polyphenol (Tannins)									
Lead acetate	+	+	+	+	+	+	-	+	+
Ferric chloride	+	+	+	+	+	+	-	-	-
Flavonoid test									
Alcoholic KOH	+	+	+	+	+	+	+	+	+
Sulfuric acid	+	+	+	+	+	+	+	+	+
Triterpenoid (Liebermann- Burchard test)	-	-	-	-	-	-	-	-	-
Peptides free amino group	+	+	+	+	+	+	+	-	-
Carbohydrate (Molish test)	+	+	+	+	+	+	+	+	+
Glycosides (Benedict)									
Before hydrolysis	+	+	+	+	+	+	+	+	+
After hydrolysis	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	-	-	-	-
Protein (Biuret test)	-	-	-	-	-	-	+	-	-
Polyphenol (Tannins)									
Lead acetate	+	+	+	+	+	+	-	+	+
Ferric chloride	+	+	+	+	+	+	-	-	-

+ =The extract contain the designated phytochemicals. ; - =The extract does not contain the designated phytochemicals.

Table 3. Thin layer chromatography for glycosides (R t and color of spots).

Compound		Rate of flow (Rf)	Color of spot
Glycosides	FI	0.8	Brown
	FII	0.187	Brown

Table 4. The main functional groups and their frequencies in FTIR of fraction I and fraction II.

Band frequency (cm^{-1})		Group	Mode of vibration	Functional group
FI	FII			
3423 (br)	3411 (br)	-OH	Stretch	Hydroxyl
2927	2927	C-H	Stretch	CH3
1724	1724	C=O	Stretch	Carboxyl group
1602	1614	C=C	Stretch	Ring
1515	1573	C=C	stretch	Ring
1452	1423	C-H	Asymmetric bending	CH3
1384	-	-OH	Bend in plane	Hydroxyl
1272	1261	C-O-C	Asymmetric stretch	Ether
1070	1076	C-O-C	Symmetrical stretch	Ether group

Table 5. Mean ± SE for the effect of different concentrations of (SM, SA, LM, RM, RA, LA, glycoside (FI) and proteoglycan extracts of *C .arvensis* on the proliferation of RD tumor cell line after 24 h treatments *in vitro* (observations of OD).

Extracts	Concentration mg/ml								
	0	0.078	0.156	0.312	0.625	1.25	2.5	5	10
Stem methanolic	0.739±0.001	0.741±0.003	0.739±0.017	0.734±0.007	0.736±0.01	0.734±0.025	0.645±0.009	0.599±0.006	0.588±0.012
Stem aqueous	0.739±0.004	0.744±0.028	0.737±0.003	0.725±0.003	0.718±0.006	0.558±0.005	0.549±0.01	0.550±0.002	0.546±0.018
Proteoglycan	0.739±0.021	0.746±0.008	0.739±0.002	0.735±0.017	0.727±0.0005	0.719±0.003	0.643±0.0005	0.582±0.0005	0.580±0.013
Leaves methanolic	0.794±0.001	0.793±0.003	0.778±0.002	0.778±0.001	0.776±0.0008	0.772±0.006	0.608±0.0005	0.592±0.004	0.228±0.034
Root methanolic	0.794±0.001	0.794±0.003	0.786±0.001	0.782±0.001	0.762±0.001	0.751±0.002	0.731±0.002	0.729±0.0005	0.728±0.005
Root aqueous	0.794±0.001	0.786±0.001	0.784±0.005	0.775±0.014	0.771±0.001	0.7630±0.001	0.760±0.003	0.7590±0.001	0.621±0.009
Leaves aqueous	0.765±0.005	0.759±0.003	0.757±0.001	0.756±0.001	0.751±0.004	0.749±0.005	0.707±0.0005	0.552±0.002	0.225±0.03
Glycoside	0.747±0.003	0.751±0.001	0.731±0.002	0.227±0.0005	0.225±0.0001	0.221±0.001	0.219±0.002	0.206±0.001	0.201±0.048

Effectors	Extracts	Concentration	Extract and concentration
LSD (0.05)	0.006629	0.007031	0.019886

SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

After 72 h, proteoglycan and glycoside FI possessed an activity from 0.156 to10 mg/ml; their activity against the growth of RD cells increased by increasing their concentration. The results showed that glycoside FI was more effective extract against the proliferation of RD cells especially at 10 mg/ml where the value of OD was 0.105 ± 0.002 (Table 7).

The exposure times had a highly significant effect ($P \leq 0.001$) on growth of RD tumor cell line treated with stems methanol extract, stems aqueous extract, proteoglycan and leaves aqueous extracts. Leaves and roots methanol extracts had less significant effect ($P \leq 0.01$). Time was not effective significantly on growth of

Table 6. Mean ± SE for the effect of different concentrations of SM, SA, LM, RM, RA, LA, Glycoside FI and proteoglycan extracts of *C. arvensis* on the proliferation of RD tumor cell line after 48 h treatments *in vitro* (observations of OD).

Extract	Concentration mg/ml								
	0	0.078	0.156	0.312	0.625	1.25	2.5	5	10
Stem methanolic	0.803±0.005	0.787±0.031	0.749±0.041	0.584±0.043	0.575±0.021	0.557±0.018	0.399±0.036	0.390±0.011	0.209±0.008
Stem aqueous	0.803±0.005	0.795±0.009	0.767±0.027	0.458±0.0005	0.330±0.04	0.324±0.024	0.325±0.023	0.183±0.002	0.183±0.013
Proteoglycan	0.803±0.005	0.803±0.021	0.673±0.026	0.705±0.002	0.372±0.012	0.371±0.005	0.372±0.016	0.094±0.002	0.077±0.001
Leaves methanolic	0.798±0.041	0.789±0.02	0.787±0.06	0.741±0.031	0.640±0.009	0.187±0.0005	0.172±0.001	0.155±0.002	0.153±0.001
Root methanolic	0.798±0.041	0.798±0.011	0.797±0.015	0.766±0.004	0.754±0.064	0.631±0.077	0.231±0.017	0.225±0.001	0.222±0.003
Root aqueous	0.798±0.041	0.788±0.031	0.787±0.039	0.776±0.036	0.770±0.017	0.766±0.002	0.764±0.007	0.761±0.018	0.446±0.03
Leaves aqueous	0.762±0.0.052	0.458±0.015	0.461±0.03	0.458±0.027	0.483±0.018	0.412±0.004	0.416±0.006	0.386±0.022	0.208±0.018
Glycoside	0.731±0.002	0.746±0.003	0.705±0.002	0.221±0.001	0.209±0.002	0.149±0.0005	0.139±0.001	0.121±0.001	0.110±0.002

Effector	Extract	Concentration	Extracts and concentration
LSD (0.05)	0.02336	0.02478	0.07009

SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

Table 7. Mean ± SE for the effect of different concentrations of (SM, SA, LM, RM, RA, LA, Glycoside (FI) and Proteoglycan) extracts of *C. arvensis* on the proliferation of RD tumor cell line after 72 h treatments *in vitro* (observations of OD).

Extracts	Concentration mg/ml								
	0	0.078	0.156	0.312	0.625	1.25	2.5	5	10
Stem methanolic	0.756±0.028	0.756±0.00	0.436±0.015	0.317±0.023	0.225±0.018	0.201±0.014	0.216±0.014	0.216±0.004	0.156±0.0005
Stem aqueous	0.756±0.028	0.756±0.014	0.351±0.014	0.338±0.007	0.273±0.035	0.237±0.015	0.223±0.011	0.207±0.012	0.203±0.001
Proteoglycan	0.756±0.028	0.755±0.012	0.489±0.009	0.340±0.029	0.204±0.002	0.174±0.001	0.170±0.0005	0.162±0.001	0.160±0.001
Leaves methanolic	0.794±0.002	0.783±0.0008	0.779±0.005	0.679±0.002	0.521±0.002	0.164±0.001	0.144±0.002	0.141±0.001	0.133±0.002
Root methanolic	0.794±0.002	0.793±0.001	0.782±0.001	0.759±0.003	0.731±0.005	0.628±0.002	0.232±0.0005	0.158±0.001	0.157±0.002
Root aqueous	0.794±0.002	0.786±0.001	0.785±0.002	0.775±0.002	0.764±0.002	0.752±0.0005	0.697±0.002	0.571±0.002	0.196±0.001
Leaves aqueous	0.763±0.020	0.746±0.044	0.548±0.016	0.370±0.035	0.272±0.01	0.264±0.024	0.246±0.004	0.161±0.003	0.160±0.004
Glycoside	0.718±0.001	0.716±0.001	0.681±0.001	0.208±0.002	0.199±0.001	0.143±0.001	0.136±0.002	0.117±0.001	0.105±0.002

Effector	Extract	Concentration	Extracts and concentration
LSD (0.05)	0.01215	0.01288	0.03644

SM = stem methanolic, SA = stem aqueous, LM = leaves methanolic, RM = root methanolic, RA = root aqueous, LA = leaves aqueous. LSD = least significant difference.

RD cell line when subjected to roots aqueous extract and glycoside FI. Table 8 demonstrated that stems and leaves methanol extracts and proteoglycan were more toxic after 72 h than 24 and 48 h on growth of RD cells. However, stems and leaves aqueous extracts, root methanol had the same effect at 48 and 72 h on growth of these cells and all previous extracts were more effective than 24 h. Glycoside FI had CC 50% values of

Table 8. Mean ±SE for the effect of exposure time to (SM, SA, LM, RM, RA, LA, Glycoside (FI) and proteoglycan) extracts of *C.arvensis* on the proliferation of RD tumor cells *in vitro* (observation OD).

Extract	Time (h)			LSD
	24	48	72	
Stem methanol	0.695±0.005	0.561±0.038	0.364±0.043	0.0967
Stem aqueous	0.652±0.004	0.463±0.047	0.372±0.041	0.1075
Proteoglycan	0.690±0.001	0.474±0.052	0.357±0.046	0.1161
Leaves methanol	0.680±0.004	0.491±0.058	0.460±0.057	0.1440
Root methanol	0.762±0.004	0.580±0.051	0.559±0.053	0.1203
Root aqueous	0.757±0.001	0.739±0.021	0.680±0.035	-
Leaves aqueous	0.669±0.001	0.449±0.027	0.392±0.044	0.1002
Glycoside	0.392±0.002	0.348±0.053	0.336±0.051	-

SE=standard error. SM = stem methanol, SA = stem aqueous, LM = leaves methanol, RM = root methanol, RA = root aqueous and LA = leaves aqueous. LSD = least significant difference.

Table 9. Mean ± SE for MI of RD tumor cells after 72 h treatment with glycoside (FI) extract of *C. arvensis in vitro*.

Parameter	Concentration mg/ml	MI%
Control	0	10.34±0.41
Glycoside extract	0.353	1.93±0.13
	0.177	3.44±0.29
	0.088	6.71±0.68
L.S.D (0.05)	1.409	

SE = Standard error, LSD = least significant difference.

1.775, 0.870 and 0.706 mg/ml after exposure to 24, 48, and 72 h, respectively. The result of cytogenetic analysis showed a decrease in MI of RD tumor cell line treated with concentrations of 0.353, 0.177 and 0.088 mg/ml of glycoside FI extract as compared with control groups 1.93 ± 0.13, 3.44 ± 0.29, 6.71 ± 0.68 and 10.34 ± 0.41, respectively (Table 9).

DISCUSSION

Cytotoxic effect of (aqueous and methanol) crude extracts, PGM and glycoside FI of *C. arvensis* on RD tumor cell line *in vitro*

The cytotoxic effect of all extracts of *C. arvens*is on RD tumor cell line varied depending on the extract and its concentration. The results showed that glycoside extract FI was more effective against the proliferation of RD tumor cell line. This result was supported by Mojab et al. (2003) where it was shown that glycoside components play an important role in cytotoxicity against cancer. The high inhibition activity of glycoside FI extract against the RD cell line may be explained by different mechanisms. Glycoside extracts had different inhibitory properties on potassium fluxes that clearly inhibit the potassium intake of those cells by inhibition of the Na-KATPase enzyme activity, which in turn leads to significant change in the permeability of the plasma membrane that allow the entry of compounds to the cell and disrupt the nitrogen base sequence of DNA (Chakravarty, 1976). Choi et al. (1994) found the anti-mutagenic capacity of glycoside dependent on free hydroxyl groups. Merfort et al. (1994) concluded that most of glycosides penetrate into human skin, making it a candidate for prevention and treatment of skin cancer.

Koishi et al. (1992) showed that quercitin inhibited production of heat shock proteins in several malignant cell lines, including colon cancer. Heat shock proteins form a complex with mutant p53, which allows tumor cells to bypass normal mechanisms of cell cycle arrest (Ranelletti et al., 1999). The obtained results of glycoside cytotoxicity may be attributed to the inhibition in the production of heat shock proteins. The ability of PGM extract of *C. arvensis* to reduce the proliferation of RD cells was in time-dependent manner, since its activity increased

after 48 and 72 h, and its effect was started at concentrations of 0.156 µg/ml, up to 10 mg/ml. Calvino (2002) prepared a novel proteoglycan mixture (PGM) extract from C. *arvensis* and their results showed that these extracts inhibited tumor growth and angiogenesis in chick embryo and improved lymphocyte. Toxicity of *C. arvensis* may be related to the presence of several types of alkaloids (pseudotropin) or other components of flavonoids, saponins, carbohydrate (AL-Edani, 1998). Winter (2008) showed that alkaloids reduced the proliferation of mouse lymphoblast cell line. Both leaves and stems methanol extracts showed high activity against the proliferation of RD tumor cell line at concentration of 0.156 mg/ml and up to 10 mg/ml, especially at 72 h. The current results were similar to that obtained by Sadeghi-aliabadi et al. (2008) where they showed that chloroform, methanol and ethanol extracts of aerial parts of *C. arvensis* possessed high cytotoxic activity on Hela tumor cell line.

Awad et al. (2004) demonstrated that the roots contain low amounts of crude protein (18.7%), aspartic acid and alanine less than green parts and had high amounts of phenylalanine, which may explain the low activity of roots aqueous extract against the proliferation of RD cell line. Some root compounds have low ability to be absorbed by cell membranes (Marja, 2004). This low activity of root extract may be due to the resistance of the cell lines to its compounds. Lee et al. (2003) found that the tumor cells vary in their response to different drugs or crude extracts according to the types of cell membrane receptors. Our results revealed that the methanol roots extract had more toxicity than roots aqueous extract since the methanol had high polarity which could dissolve both polar and non polar components which then can be actively passed through the plasma membrane. Cannell (1998) and Rajendran and Ramakrishnan (2009) found that the high polarity of methanol extract of *Artocarpus heterophyllus* was responsible for inhibiting nearly 100% of Hep2 cells. The other explanation for the differences in cytotoxic activity between aqueous and methanol extracts may be due to the presence of the calystegin alkaloid extracted by methanol (Russel, 1993). A study of Khanavi et al. (2009) supported the obtained results where they found that methanol extract from *Stachys* species contains the most potent antioxidant and had more activity than the aqueous extract.

Cytogenetic effect of glycoside (FI) extract of *C. arvensis* on the RD tumor cell line *in vitro*

The present experiment focused on determining the effect of glycoside FI extract on MI of RD cell line. Rhabdomyosarcoma tumor cell line showed a decrease in MI when treated with all concentrations (0.353, 0.177 and 0.088 mg/ml) of glycoside FI extract as compared with untreated cells (negative control group); their effects were concentration-dependent manner. The ability of glycoside FI to reduce the MI of RD tumor cell line might be explained by its contents of chemical constituents that had ability to reduce cell cycle progression. Amorim et al. (2000) found that the decrease in MI reflects the inhibition of cell-cycle progression and/or the loss of proliferative capacity. Cell cycle arrest in S-phase was found in human lung squamous carcinoma cells treated with glycoside extract (Leung et al., 2005). Wang et al. (2007) found that the glycoside isolated from two Asian plants *Epimedium koreaonum* and *Terminalia arjuna* were traditionally used as anticancer medicines; they inhibit proliferation of MCF-7 (breast cancer) and HepG2 (liver cancer) cells in a dose-dependent manner.

Conclusion

Fraction I of glycoside caused high inhibition activity on growth of RD cell line after 24, 48 and 72 h. The aqueous roots extract had less inhibiting activity against the growth of RD cells. The Fraction I of glycoside showed high antimitotic effect on RD cell line with concentrations of 0.353, 0.177 and 0.088 mg/ml.

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Conflict of Interests

The author(s) have not declared any conflict of interests.

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