African Journal of Biochemistry Research Vol. 5(14), pp. 373-378, December 2011
Available online at http://www.academicjournals.org/AJBR
DOI: 10.5897/AJBR11.095
ISSN 1996-0778 ©2011 Academic Journals

Full Length Research Paper

The apoptotic and cytotoxic effects of *Polygonum avicular* extract on Hela-S cervical cancer cell line

Rahmati Mohammad[1], Bannazadeh Hossein[2], Fazli Davood[3]*, Tahmasebzadeh Farnaz[3], Farrokhi Ali[4] and Rasmi Yusef[5]

[1]Department of Clinical Biochemistry, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
[2]Department of Clinical microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.
[3] Department of Basic Sciences, Payame Noor University, PO BOX 19395-3697 Tehran, Iran.
[4]Royan Institute, Tehran, Iran.
[5]Department of Clinical Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.

Accepted 22 November, 2011

Cervical cancer is the second main cause of cancer leading death in women. Therefore, targeting it, preferably with natural compounds derived from medicinal plant such as Polygonum avicular as an herbal extract have astringent properties. Herbal medicines in all regions of the developing world are widely used. Unlike the heavy use, there are very little information about the health and efficiency of the treatment by these plants. On the other hand, it's proven that the relationship between apoptosis and cancer has been increased. In the present study the induction of apoptosis as well as its proliferation effects of polygonum avicular extract on Hela-S cervical cancer cell line has been investigated. First, total extract of Polygonum avicular was prepared by n-hexane, methanol and dichloromethane. Then, cytotoxic effect of methanolic phase was studied on Hela-S cervical cancer cell line with 12, 24 and 48 h MTT assays. Finally, after determination of IC_{50}, cells were treated with methanolic extract and Hoechest staning assay was done to measure the amount of apoptosis by the extract. Data analysis showed that methanolic extract of Polygonum avicular has dose-dependent cytotoxic effect on Hela-S cervical cancer cell line with IC_{50}= 0.27±0.07 -0.41±0.02 mg/ml and the extract causes apoptosis with time-dependent manner. In conclusion, methanolic extract of polygonum avicular has cytotoxic and apoptotic effect on Hela-S cervical cell line and may be exploited as potential source for developing novel drugs against cervical cancer.

Key words: Cervical cancer, polygonum avicular, Hela-S cervical cancer cell line, apoptosis.

INTRODUCTION

Cervical cancer is the second main cause of cancer leading death in women. Approximately 1 in 135 females will develop invasive cervical cancer in their lifetime (Alberta Health Services, 2011). In 2006, about 500 thousand new cases of cervical cancer have been reported and are forecasted about 280,000 deaths of it while most of these patients live in developing countries (Castellsague et al., 2006).

Human papilloma virus (HPV) is the main cause of cancers of genital. More than 99% of cervical cancer cases are related to infection of genital HPV with the peak rate in adolescents and young adults less than 25 years. Despite reports between international field of various screening, diagnosis and treatment of cervical cancer, statistics of incidence and mortality of cervical cancer are not known due to the lack of cancer registration network in Iran. According to the Institute of Cancer Registration, uterine and cervical cancer incidence is about 7 per 100 thousand. This cancer typically occurs between 30 to 55 years old, but recently several reports of younger women are published (Behtash et al., 2003; Nazari et al., 2007).

Herbal medicines in all regions of the developing world are widely used. Unlike the heavy use, there is too little information about the health and efficiency of the

*Corresponding author. E-mail: rahmati_bio@yahoo.com. Tel:/Fax.: +98-4113364666.

treatment by these plants. It has been shown that some medicinal plants have anti-cancer activity. But lack of scientific evidence in connection with their mechanism of action reduced their clinical applications (Cassileth, 1999). Polygonum avicular is a member of Polygonaceae - Dock family. This plant has spread in Australia, Europe, South Africa, Mediterranean, North Africa, Middle East and India. This plant has astringent properties and is used for treatment of diarrhea, stop bleeding and healing it. This plant is a diuretic and also used for treatment of urinary retention and for excretion of kidney stones (Lazarides et al., 1997). It has been proven that this plant has large amounts of antioxidants. According to this fact that cancer is linked with large amounts of free radicals, we can expect its positive effects on cancer controlling (Castellsague et al., 2006). Unfortunately, there is a little information about the health and efficiency of treatment with this plant. The clinical usage of avicular can be reduced by lack of scientific evidence in relation to routes (Cassileth, 1999).

It's proven that the relationship between apoptosis and cancer has been increased (Bold et al., 1997; Hsu et al., 2005). Anti-cancer activity of some therapeutic substances is involved in the induction of apoptosis, which can be used to cancer control (Li et al., 2005; Hsu et al., 2005). The Hela-S cervical cancer cell line is used as a suitable model for studying cervical cancer and apoptosis. In the present study apoptotic and proliferative effects of polygonum acivular extract have been investigated on cervical cancer cell line.

MATERIALS AND METHODS

Herbal extraction preparation

The effect of Polygonum avicular extracts on Hela-S cervical cancer cell apoptosis is determined after collecting and selection of plant shoots. They were dried in suitable place away from sunlight and then dried shoots were pround by mechanical grinder. Then, 100 g powder was dissolved in 200 ml n-hexane and solution was shaken for 4 hours at 45°C. Then, supernatant was transferred to a tube. In the next step, residue of n-hexane extraction was dissolved in 200 ml dichloromethane instead of n-hexane and same steps repeated using dichloromethane. In the next step, debris of previous extraction step was dissolved in 200 ml methanol and at the same way supernatant was collected. After that, fractions were dried completely by the rotatory evaporator at 45°C with low pressure and were frozen at -20°C until use.

Cell culture and MTT assay

Hela-S cell line was incubated in RPMI-1640 medium (Gibco, Invitrogen, UK) contain 10% heat-inactivated fetal bovine serum (Gibco, Invitrogen, UK), 2 mg/ml sodium bicarbonate, 0.05 mg/ml penicillin G (Serva Co, Germany), 0.08 mg/ml streptomycin (Merck Co, Germany) at incubator with 37°C and 5% CO_2. Then they treated with different concentration of extract (0, 0.005, 0.05, 0.01, 0.025, 0.075, 0.1, 0.125, 0.15, 0.175, 0.2, 0.25, 0.3, 0.35, 0.5 mg/ml) at different times (12, 24 and 48 h). Polygonum avicular methanolic extract in different concentrations was dissolved in dimethysulfoxide (DMSO) then was used for treating on cervical cancer cells. At this study extract effect on cell growth and apoptosis was checked. Also, cell in culture medium RPMI 1640/DMSO was used as a control. Briefly, 1000 cell/well were cultivated in a 96 well plate. After incubation cells were treated with different concentrations of mathanolic extract of avicular in the quadruplicate manner. After incubation, the medium of all wells of plate was exchanged with fresh medium and cells were left for 24 h in incubator. Then, the medium of all wells was removed carefully and 50 µl of 2 mg/ml MTT (Sigma Co, Germany) dissolved in phosphate buffer saline (PBS) was added to each well and incubated for 4 h. After removing of well content, 200 µl DMSO was added to wells. Immediately absorbance of each well was read in 570 nm using 630 nm as reference wavelength.

Apoptosis

Cultured cells were analyzed for apoptosis by using Hoechest staning after treating with different doses of extract at times 12, 24 and 48 h. After treating, cells were fixed by methanol and stained with Hochest solution then evaluated with fluorescent microscopy.

Data analysis

For data analysis of MTT assays, mean optical density (OD) of each well was calculated and percentages were calculated by use of control OD as a reference. Then the graph of results was plotted by SPSS 16.0. In apoptosis, cells were counted with intact nucleus and degraded nucleus under fluorescent microscope.

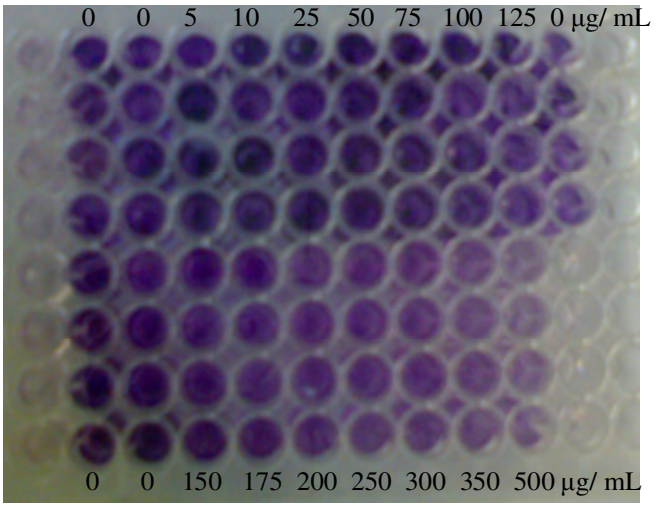
RESULT

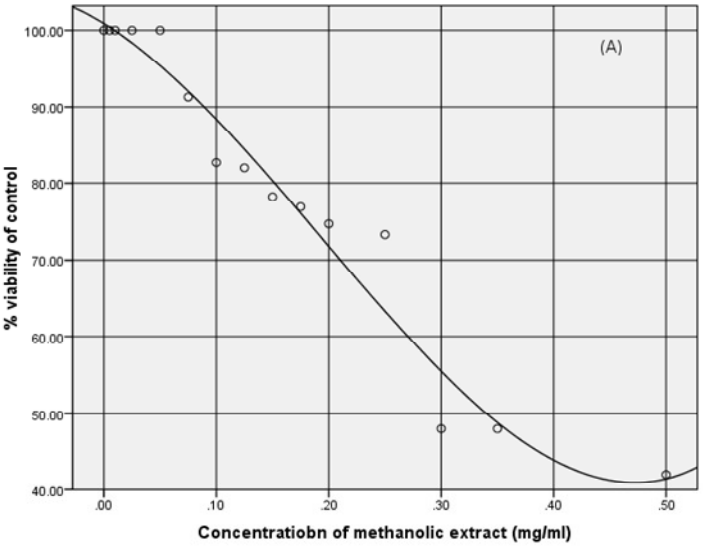
The effect of Polygonum avicular extracts on Hela-S cervical cancer cell apoptosis and cytotoxicity was demonstrated. Data analysis of cytotoxicity assay showed that IC_{50} of methanolic extract of Polygonum avicular on Hela-S cervical cancer cell line is 0.34 ± 0.03mg/ml, 0.27 ± 0.07 mg/ml and 0.41 ± 0.02 mg/ml for 12, 24 and 48 h MTT assays, respectively. The IC_{50} of methanolic extract of Polygonum avicular on Hela-S cervical cancer cell line is dose-dependent and time dependent (Figure 1). Therefore, we investigated apoptotoic effect of Polygonum avicular on Hela-S cervical cancer cell line for 12, 24, and 48 h exposure time. Morphological analysis showed significant difference between control and treated cells.

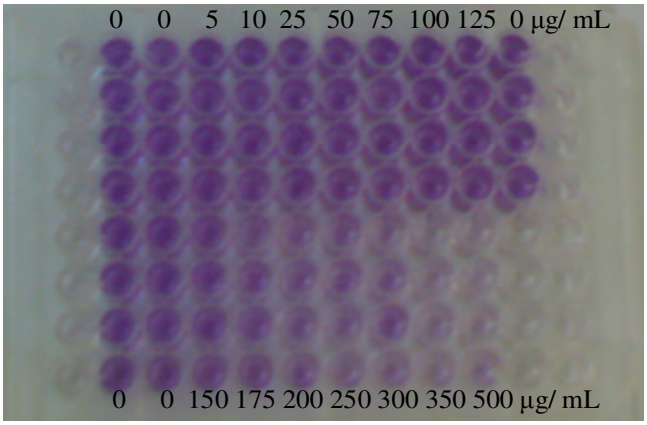
Data analysis obtained from apoptosis assay showed that methanolic extract of Polygonum avicular causes apoptosis time-dependently. The time of 24 h treatment completely cause apoptosis (Figures 2 and 3). In addition, in this time apoptosis was 46.30 folds in the Hela-S cell line treated with methanolic extract of Polygonum avicular in comparison with untreated cells. Therefore, the time of 24 h may be a convenient treating time for making apoptosis by methanolic extract of Polygonum avicular in Hela-S cell line.

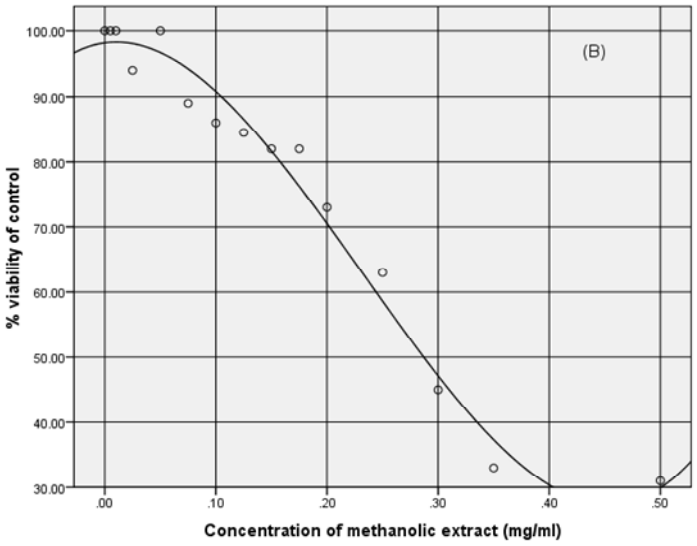
DISCUSSION

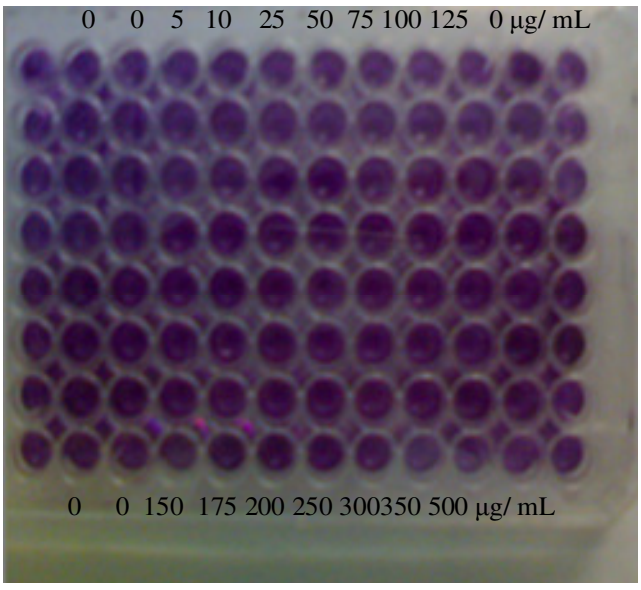
In the present study, total extract used instead of pure components of Polygonum avicular extract. Because,











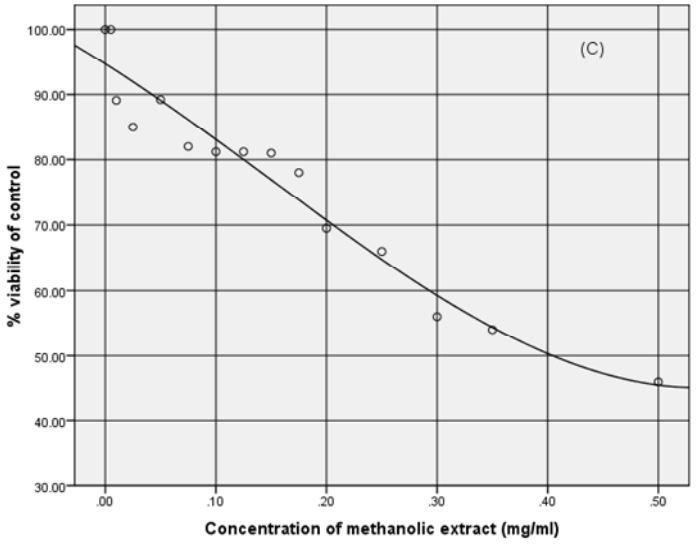


Figure 1. Cytotoxic effect of methanolic extract of Polygonum avicular on Hela-S cervical cancer cell line after 12 h (A), 24 h (B) and 48 h (C) exposure and IC50 of methanolic extract of Polygonum avicular on Hela-S tumor cell line after 12, 24 and 48 h treatment.

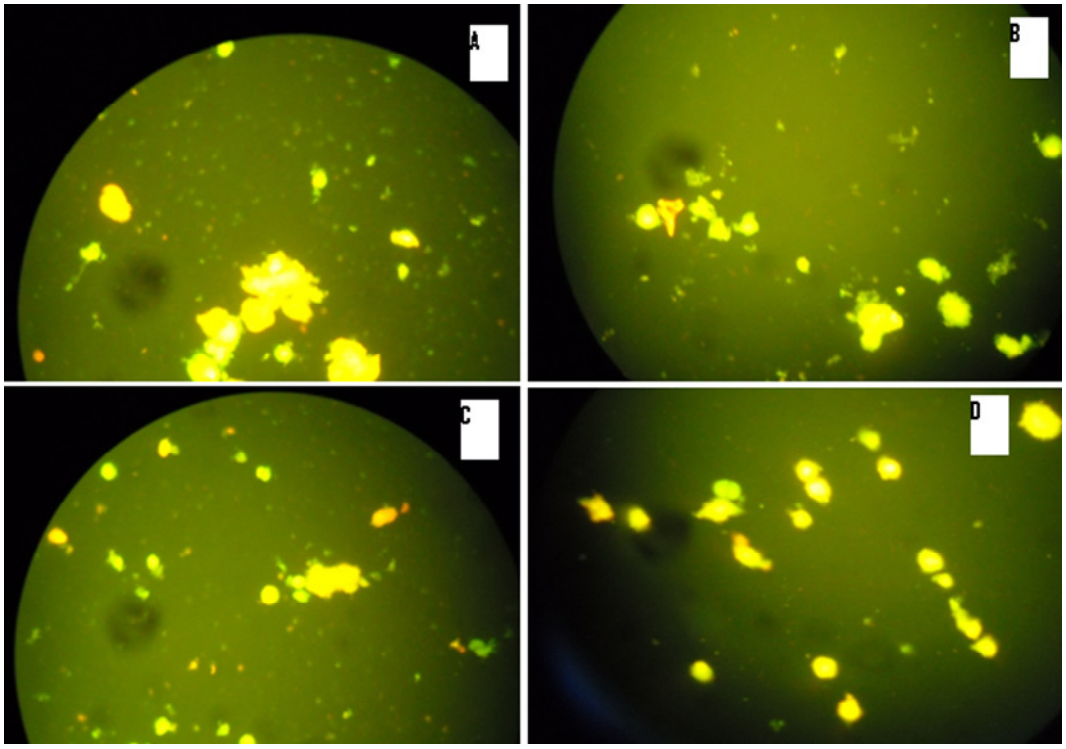


Figure 2. Representative Hoechst staining of Hela-S cervical cancer cell lines at baseline (A), 12 h (B), 24 h (C) and 48 h (D) after treatment with Polygonum avicular extract.

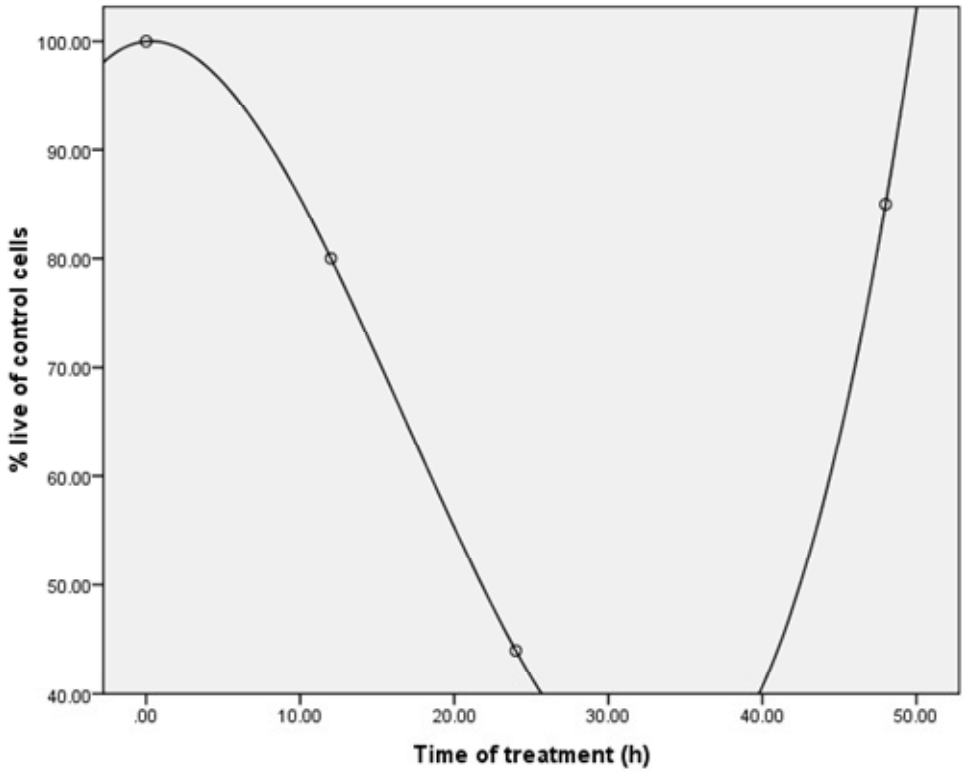


Figure 3. Correlation between different treating times of methanolic extract of Polygonum avicular and apoptosis in Hela-S cell line.

there is no study about the pure component. According to Chen and Shi (2009) report, extraction of pure Polygonum avicular component is time consumable and needs to HPLC.

In the current work, MTT assay showed that methanolic extract of Polygonum avicular has dose and time-dependent cytotoxicity on the Hela-S cervical cancer cell line (IC_{50} = 0.27 to 0.41 mg/ml). This finding is in accordance with the results of Manoharan et al., (2007) in which Polygonum bistorta (Polygonaceae) was evaluated for their cytotoxic activity against P338 (Murine lymphocytic leukaemia), HepG2 (Hepatocellular carcinoma), J82 (Bladder transitional carcinoma), HL60 (Human leukaemia), MCF7 (Human breast cancer) and LL2 (Lewis lung carcinoma) cancer cell lines in culture. In addition, results of another study showed protective effects of preconditioning human umbilical vein endothelial cells (HUVECs) with Polygonum multiflorum stilbeneglycoside (PMS) under anoxia/reoxygenation (A/R), PMS incubation attenuated A/R-induced injury in a concentration-dependent manner (Liu et al., 2010). There is no study with Polygonum avicular about cytotoxicity and the current study is the first one on Hela-S cervical cancer cell line. Therefore, there is a need for further study of Polygonum avicular extraction constituents in the future. However, results of current work demonstrated that IC_{50} of Polygonum avicular extract is between 0.27 ± 0.07 - 0.41 ± 0.02 mg/ml in Hela-S cervical cancer cell line. This different finding may be due to different behavior of cell lines in response to Polygonum avicular and shows that these cancers may differently response to exposure with Polygonum avicular *in vivo*.

Apoptotic effects of methanolic extract of Polygonum avicular showed that it has time-dependent apoptotic effect on the Hela-S cervical cancer cell line. This finding is in accordance with the result of Shin et al. (2011) study in which the methanol extract of P. cuspidatum (MEPC) inhibited the proliferation of oral cancer cells by inducing caspase-dependent apoptosis.

In addition, results of another study showed apoptotic effects of tryptanthrin, an active ingredient of Polygonum tinctorium Lour, on HL-60 (Kimoto et al., 2001). There is no study with Polygonum avicular about apoptotic effects and the current study is the first one on Hela-S cervical cancer cell line.

Currently, Papanicolaou (Pap) test is the first line of cervical cancer screening and there are different types of standard treatment methods for cervical cancer patients such as surgery, radiation, chemotherapy, laser therapy, HPV vaccines, and gene targeted therapy (Garland and Smith, 2010). However, there are harsh side effects about all (Barnas et al., 2011). Therefore, with regarding to these undesirable effects and result of the current study, it seems that Polygonum avicular may be an appropriate source for developing novel drugs against cervical cancer and can be a safe alternative for current treatment regimens.

In summary, the results of the current study showed that methanolic extract of Polygonum avicular has inducing effect on apoptosis in the Hela-S cervical cancer cell line. Cytotxicity and apoptotic effects on Hela-S cervical cancer cell line by methanolic extract of Polygonum avicular are reported for the first time and even any study has not been reported to date. However, similar works on other cell lines with other Polygonum extractions have been performed (Zhang et al., 2010; Brandao et al., 2010). The results of these studies have showed that Polygonum extract has cytotoxic and apoptotic effects on cervical cancer. Because based on our work methanolic extract of Polygonum avicular, induced apoptosis time-dependently in Hela-S tumor cell lines. Therefore, it can have potentially apoptotic compounds, especially for exploiting in treatment of cervical cancer.

Conclusion

In conclusion, current work demonstrated that methanolic extract of Polygonum avicular has potent anti-growth effect on Hela-S cell line and time-dependently induces apoptosis in this cell line as in vitro model of cervical cancer. Therefore, Polygonum avicular can be a natural potent chemopreventive and chemotherapeutic plant for patients with cervical cancer and constituents of its methanolic extract can be an appropriate candidate for drug development.

ACKNOWLEDGEMENTS

We thank thereby Tabriz Payam Noor University and Tabriz University of Medical Sciences for funding this research and for supplying technical support.

REFERENCES

Alberta Health Services (2011). 2008 Report on Cancer Statistics in Alberta.

Barnas E, Skret-Magierlo J, Skret A, Bidzinski M (2011). The quality of life of women treated for cervical cancer. Eur. J. Oncol. Nurs., [Epub ahead of print].

Brandao GC, Kroon EG, Duarte MG, Braga FC, de Souza Filho JD, de Oliveira AB (2010). Antimicrobial, antiviral and cytotoxic activity of extracts and constituents from Polygonum spectabile Mart. Phytomedicine, 17(12): 926-929.

Behtash N, Mousavi A, Mohit M, Modares M, Khanafshar N, Hanjani P (2003). Simple hysterectomy in the presence of invasive cervical cancer in Iran. Int. J. Gynecol. Cancer, 13: 177-181.

Bold RJ, Termuhlen PM, McConkey DJ (1997). Apoptosis, cancer and cancer therapy. Surg. Oncol., 6(3): 133-142.

Cassileth BR (1999). Alternative and complementary medicine. Cancer, 86(10): 1900-1902.

Castellsague X, Diaz M, Sanjose S (2006). Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. J. Natl. Cancer Inst., 98(5): 303-315.

Chen J, Shi Y (2009). Determination of quercetin and kaempferol in

Polygonum aviculare by HPLC. Zhongguo Zhong Yao Za Zhi, 34(4): 423-427.
Garland SM, Smith JS (2010). Human papillomavirus vaccines: current status and future prospects. Drugs, 70(9): 1079-1098.
Hsu YL, Kuo PL, Lin LT, Lin CC (2005). Asiatic acid, a triterpene, induces apoptosis and cell cycle arrest throught activation of extracellular signal-regulated kinase and p38 mitogen-activated protein kinase pathways in human breast cancer cells. J. Pharmacol. Exp. Ther., 313(1): 333-344.
Kimoto T, Hino K, Koya-Miyata S, Yamamoto Y, Takeuchi M, Nishizaki Y, Micallef MJ, Ushio S, Iwaki K, Ikeda M, Kurimoto M (2001). Cell differentiation and apoptosis of monocytic and promyelocytic leukemia cells (U-937 and HL-60) by tryptanthrin, an active ingredient of Polygonum tinctorium Lour. Pathol. Int., 51(5): 315-325.
Lazarides M, Cowley K, Hohnen P (1997). Handbook of Australian Weeds. CSIRO publishing.
Li Y, Ahmed F, Ali S, Philip PA, Kucuk O, Sarkar FH (2005). Inactivation of nuclear factor kappaB by soy isoflavone genistein contributes to increased apoptosis induced by chemotherapeutic agent in human cancer cells. Cancer Res., 65(15): 6934-6942.
Liu LP, Liao ZP, Yin D, Li WD, Liu D, Li Q, Huang QR, Yang YF, He M (2010). The protective effects of Polygonum multiflorum stilbeneglycoside preconditioning in an ischemia/reperfusion model of HUVECs. Acta Pharmacol. Sin., 31(4): 405-412.
Manoharan KP, Yang D, Hsu A, Huat BT (2007). Evaluation of Polygonum bistorta for anticancer potential using selected cancer cell lines. Med. Chem., 3(2): 121-126.
Nazari Z, Behtash N, Gilan MM, Ganjoei TA (2007). Cervical carcinoma simulating advanced ovarian cancer. Eur. J. Surg. Oncol., 33(1): 123-124.
Shin JA, Shim JH, Jeon JG, Choi KH, Choi ES, Cho NP, Cho SD (2011). Apoptotic effect of Polygonum Cuspidatum in oral cancer cells through the regulation of specificity protein 1. Oral Dis., 17(2): 162-170.
Zhang RC, Liu B, Sun ZX, Xu DY (2010). Effects of extract of Polygonum multiflorum on cell cycle arrest and apoptosis of human liver cell line L02. Zhong Xi Yi Jie He Xue Bao, 8(6): 554-561.