

The Effects of Konar Extract on Kidney Cell Line in Cell Culture

Jamshidpoor A., Ahmadi R., Mazloomifar H.*, and Mahdavi E.

Abstract— There are reports indicating that some plant extracts can affect on cell proliferation in cell lines *in vitro*. This study was exerted to determine the effects of Konar (*Ziziphus Spina Christi*) extract on viability of embryonic kidney (HEK) cell line. In this laboratory experimental study, HEK cell line was divided into 6 groups including control, and groups receiving *Ziziphus Spina christi* extract in 10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml and 0.001mg/ml doses. After 48 hours the viability of cell line was measured using MTT assay. The data was analyzed using ANOVA. Our findings show that viability of HEK cell line decreased significantly only in groups exposed to 10mg/ml of extract ($P<0.05$). The results show that low doses of *Ziziphus Spina christi* have no significant effects on proliferation of HEK cells in cell culture, according to which, it is speculated that the low doses of the extract have no impairing effects on normal cells .

Index Terms--- Konar, Viability, HEK.

I. INTRODUCTION

HEK 293 cells that stands for human embryonic kidney cells are specific cell line originally isolated from human embryo kidney cells (from an aborted human embryo) , grown in tissue culture. This particular line was initiated by the transformation and culturing of normal HEK cells with sheared adenovirus 5 DNA. The transformation resulted in the incorporation of approximately 4.5 kilobases from the viral genome into human chromosome 19 of the HEK cells. These cells are popular for their ease of growth and transfection , making them a common cell culture in biological research . And their high transfection efficiency help to produce exogenous proteins or viruses for pharmaceutical and biomedical researches. HEK 293 cells are useful for many transfection experiments, and particularly the propagation of adenoviral-based and retroviral-based vectors. Embryonic kidneys are heterogeneous mix of almost all the types of cells present in the body . In fact the cells may be neuronal in origin , although most cells derived from an embryonic kidney would be endothelial, epithelial or fibroblasts . Neuronal origin is suspected because of the presence of mRNA and gene products that typically found in neurons[1]-[4].

Ashraf Jamshidpour (MSc) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (e-mail: Drrahamadi@yahoo.com).

Hila Mazloomifar *(corresponding author) is with the Department of Sciences and Modern Technology, Islamic Azad University of Pharmaceutical Sciences (IAUPS), Tehran, Iran , (e-mail: hilamazloomifar@yahoo.com)

Rahim Ahmadi (PhD) is with the Department of Physiology, Faculty of Basic Sciences, Islamic Azad University, Hamedan Branch, Hamedan, Iran. (e-mail: Drrahamadi@yahoo.com).

Edris Mahdavi (PhD), Department of Gardening, Faculty of Agriculture, Karaj Branch, Islamic Azad University, Karaj, Iran (Edrismahvadi@yahoo.com)

Nowadays plants extracts have attracted the researcher's attention because of their significant impacts on organs, tissues and cells. *Z. spina-christi* L., commonly known by the Persian name, “Konar” or “Sedr”, is an armed shrub or tree, which widely growth in the southern of Iran. *Ziziphus spina-Christi* known as christ's thorn is a native plant that grows in tropical and subtropical regions specially in Middle East. It belongs to the Rhamnaceae family in the order of Rosales containing about 60 genera and more than 850 species. The plant fruit is edible sweet drupe and its leaves have been used as stomachic, emollient, antiulcer, disinfectant and antifungal in the Iranian traditional medicine.[5],[6] The antibacterial, antiviral and antidiabetic effects of the extracts or fractions of the leaves of this plant has been demonstrated.[7],[8]. The cytotoxic effects of *Z. spina-christi* on cancer cells have been shown in recent studies [9]. *Zizyphus spina-christi* extract protects against aflatoxin B1-initiated hepatic carcinogenicity. [10] This study was exerted to determine the effects of Konar extract on viability of HEK cell line.

II. MATERIAL AND METHODS

A. Extract preparation

Ziziphusspina christi extract was prepared and different concentrations of extract (10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml and 0.001mg/ml) were used in our study.

B. Protocol of Study

We used MTT assay in this work to determine the effects of *Xanthium strumarium* extract on L929 cells viability in cell culture. Briefly, the procedure was carried out in the following steps:

DAY ONE: 100 μ l of cells was added into each well (96 well plate) and incubate at 37 with 5% co2 overnight.

DAY TWO: The media was removed and extract was added and incubated at 37 with 5%co2 overnight. For control 10%FBS was added to media.

DAY THREE: extract was removed from media. 20 μ l of 5 mg/ml MTT was added to each well and incubated for 4 hours at 37oC. 150 μ isopropanol was added and covered with tinfoil and agitate cells on orbital shaker for 15 min. Absorbance was read at 570 nm with a reference filter of 630 nm and recorded.

C. Statistical Analysis

Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS 19. Differences with $P<0.05$ were considered significant.

III. RESULTS

Figure I shows the viability of HEK cells in response to different doses of *Ziziphusspina christi* extract.

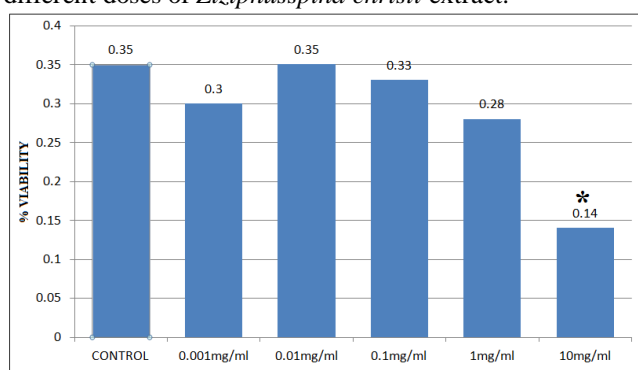


Fig I. Viability of HEK cells in response to different doses of *Ziziphusspina Christi* extract. * indicates significant difference ($P < 0.05$) compared with control group.

The results of the present study show that viability of HEK cell line decreased significantly only in group exposed to 10 mg/ml dose of extract compared with control group, but other concentrations could not significantly influence cell viability.

IV. DISCUSSION

The results show that low doses of *Ziziphus- spina christi* have no significant effects on proliferation of HEK cells in cell culture. In other words, low doses of *Ziziphus- spina christi* have not cytotoxic effects on HEK cells.

The studies show that *Z. spina-Christi* extract has anti-cancer effects and induces significant inhibition of DNA synthesis. Moreover, the results indicate that treatment of the cells with *Ziziphus-spina christi's* extract induces apoptosis [11]. The effects of *Ziziphus jujuba* fruit (ZJ) extract on the pharmacokinetics of phenacetin, a typical substrate of a cytochrome P450 enzyme CYP 1A2, also has been demonstrated in rats [12]. *Ziziphus spina-christi* protects against carbon tetrachloride-induced liver fibrosis in rats. [13] The studies also have shown the insulinotropic and subsequent hypoglycemic effects of *Ziziphus spina-christi* leaves which may be due to a sulfonylurea-like activity. [14] Despite various effects of *Ziziphus spina-christi* on different tissues and particularly on cancer cells, in our study, low doses of the extract did not show cytotoxic effects on normal kidney cell line, indicating that the extract can be used as a drug against tumors without cytotoxic effects on normal cells. However, further researches are required to confirm that *Ziziphus-spina christi* extract has no cytotoxic effects on other normal cells.

V. CONCLUSION

The results show that low doses of *Ziziphusspina christi* have no significant effects on proliferation of HEK cells in cell culture, according to which, it is speculated that the low doses of the extract have no impairing effects on normal cells.

ACKNOWLEDGMENT

We appreciate all who helped us to exert the present study.

REFERENCES

- [1] Siqueira-Silva J, Yeda FP, Favier AL, Mezin P, Silva ML, Barrella KM, et al. Infection kinetics of human adenovirus serotype 41 in HEK 293 cells. Mem Inst Oswaldo Cruz. 2009 Aug;104(5):736-44 <http://dx.doi.org/10.1590/S0074-02762009000500013>
- [2] Thomas P, Smart TG. HEK293 cell line: a vehicle for the expression of recombinant proteins. J Pharmacol Toxicol Methods. 2005 May-Jun;51(3):187-200 <http://dx.doi.org/10.1016/j.vascn.2004.08.014>
- [3] Şalva E, Turan SÖ, Ekentok C, Akbuğa J. Generation of stable cell line by using chitosan as gene delivery system. Cytotechnology. 2015 Jul 2. [Epub ahead of print] https://en.wikipedia.org/wiki/HEK_293_cells
- [4] Abdel-Wahhab MA, Omara EA, Abdel-Gali MM, Hassan NS, Nada SA, Saeed A, et al. A *Zizyphus Spina-Christi* extract protects against aflatoxin B1-Initiated hepatic carcinogenicity. Afr J Tradit Complement Altern Med. 2007;4:248–56. [PMC free article] [PubMed]
- [5] Shahat AA, Pieters L, Apers S, Nazeif NM, Abdel-Azim NS, Berghe DV, et al. Chemical and biological investigations on *Zizyphus spina-christi* L. Phytother Res. 2001;9:593–7. [PubMed] <http://dx.doi.org/10.1002/ptr.883>
- [6] Amal H, Eman IA. Effect of *Zizyphus* leaves extract on mice suffering from ehrlich ascites carcinoma. Nature Science. 2010;8:234–44.
- [7] Saied SA, Gebaur J, Hammer K, Buerkert A. *Zizyphus spina-christi* (L.) Willd: A multipurpose fruit tree. Genet Resour Crop Evol. 2008;55:929–37. <http://dx.doi.org/10.1007/s10722-007-9299-1>
- [8] Jafarian A, Zolfaghari B, Shirani K. Cytotoxicity of different extracts of arial parts of *Zizyphus spina-christi* on HeLa and MDA-MB-468 tumor cells. Adv Biomed Res. 2014 Jan 24;3:38 <http://dx.doi.org/10.4103/2277-9175.125727>
- [9] Abde-Galil MM ,Hassan Ns , Nada SA , Saeed A , el-sayed MM . *Zizyphus spina-christi* extract protects against aflatoxin B1-initiated hepatic carcinogenicity. Curr Drug Targets. 2011 Oct 1; 12(11): 1595–1653.
- [10] Ronald Ross Watson, Victor R. Preedy. Bioactive Foods and Extracts: Cancer Treatment and Prevention. CRC Press, Nov 11, 2010 - 663 .
- [11] Jing XY, Peng YR, Wang XM, Duan JA. Effects of *Zizyphus jujuba* fruit extracts on cytochrome P450 (CYP1A2) activity in rats. Chin J Nat Med. 2015 Aug;13(8):588-94. [http://dx.doi.org/10.1016/s1875-5364\(15\)30054-6](http://dx.doi.org/10.1016/s1875-5364(15)30054-6)
- [12] Amin A, Mahmoud-Ghoneim D. *Zizyphus spina-christi* protects against carbon tetrachloride-induced liver fibrosis in rats. Food Chem Toxicol. 2009 Aug;47(8):2111-9. <http://dx.doi.org/10.1016/j.fct.2009.05.038>
- [13] Abdel-Zaher AO, Salim SY, Assaf MH, Abdel-Hady RH. Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves. Ethnopharmacol. 2005 Oct 3;101(1-3):129-38. <http://dx.doi.org/10.1016/j.jep.2005.04.007>