# The Effect of Hydroalcoholic Extract of Junipers communis on Proliferation BHK Cells

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**Abstract:** Nowadays, use of medicines from plant source increases significantly with conventional therapies. Juniper communis has been observed in a wide range of traditional medicinal systems as anticancer activities an appetite suppressant, diuretic, anti-inflammatory and analgesic agents, as well as for the treatment of wounds, renal disorders gastric used. This study examines the impact of juniper extracts on the viability of BHK cell line, which is considered as normal cells. BHK cell lines were divided into 6 groups including control, and groups receiving juniperus communis extract in 10mg/ml, 1mg/ml, 0.1mg/ml, 0.01mg/ml and 0.001mg/ml doses. After 48 hours the viability of cell lines was examined with MTT assay. The data was analyzed using ANOVA. Our findings show that , in Low doses of the extract on cell line BHK, had no effect and the increase in concentration, inhibit the proliferation of cells is) on 1 mg/ml and higher doses is toxic effect). The results show that there is a need to establish a safe dose for use of these plant extracts.

Keywords: Juniperus communis, viability, BHK cell.

## 1. Introduction

Since ancient time's medicinal plants have been used to treat different ailments due to their accessibility, availability, inherited practice, economic feasibility, and perceived efficacy. Nowadays, use of medicines from plant source increases significantly with conventional therapies. Hence, the plants are gaining more attention by the researchers to find out new and effective agents for different diseases.[1] Many traditional healing herbs and plant parts have been shown to have medicinal value, and that these can be used to prevent, alleviate or cure several human diseases. The WHO estimates that more than 80% of the world's population rely either solely or largely on traditional remedies for health care.[2] Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems.Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer [3].

Juniperus communis L. subsp. hemisphaerica (Presl) Nyman (Cupressaceae) is an evergreen and dioecious shrub which grows widely in Europe, Caucasus, Turkey and Iran.[4] communis produces astringent blue-black seed cones, commonly known as juniper berries. These are used as culinary components by a variety of cultures in the regions in which they occur. The berries are used to flavour meats and sauces and as the flavouring agent for the alcoholic beverage gin. They have also been used in several traditional medicine systems. J. Communis berries are used in traditional Turkish medicine as a diuretic, as well as for gastrointestinal problems and as a general antiseptic. They are also used for the treatment of rheumatism, arthritis and gout and are believed to have both anti-inflammatory and analgesic effects. Native Americans used J.communis berries as an appetite suppressant and in the treatment of diabetes. Indeed, hypoglycemic/anti-diabetic effects have been demonstrated

in several laboratory studies[5] This plant has a number of medicinal properties and has been reported to have antimicrobial, anti-rheumatic, analgesic and contraceptive effects[4] In studies, compounds with medicinal properties are derived from the oil of juniper fruit (Including limonene, which is known as a material has strong antiviral properties. And the use of carcinogenic substances can slow tumor growth. [6].

The BHK21 cell line was established in 1961 from the kidneys of 5 Syrian hamsters from litter number 21. Since this time, this cell line has been a laboratory standard for the growth of countless viruses and the study of many biological processes. [7].

Considering that J. communis berries have been observed in a wide range of traditional medicinal systems. [5] This study examines the impact of juniper extracts on the viability of BHK cell line, which is considered as normal cells.

## 2. Material and Method

Juniper shrub and herb plants are permanent. In northern Iran were collected in late summer. And in the shade in natural conditions at a temperature of 24 to 26 C°during the 7 to 8 days were dry, Then dried herb powder and 100 grams of powder in 300 ml of solvent hydroalcoholic 50% (500 cc 96% ethanol with 500 ml of distilled water) for 24 hours was steeped Then Soxhlet was extracted with extracts obtained by the heat 50 to 40 degrees Celsius during the two or three dry days. in the end, extracts obtained after 24 hours of incubation at 37 ° C was placed and perfectly water evaporation and dry. in the next step, extract weighed and solvent pBS was dissolved and the solution 100 mg g per ml was prepared as a reservoir.juniper communis extracts were prepared and different concentrations of extract (10 mg / ml, 1 mg / ml, 0.1 mg / ml, 0.01 mg / ml, 0.001 mg / ml were used in our study.[8],[9].

In this study, Bhk cells, fibroblasts and normal cells that have been isolated from hamster kidney. BHK cell lines into three groups exposed to low-dose, medium and high plant extractor of Juniper communis. Consequently, with regard to adequate culture medium for the cells and also repeated at least six times extracts, were added to the wells. Plates were kept in incubator for 12, 24 and 48 hours. Following the passage of time the liquid to drain from the plates and color MMT was added. Looking for 4 to 6 hours after the addition of color, added a solution of DMSO and After dissolving completely, The optical density of the solutions. Using 360 nm and 570 nm was read.Statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS. Differences with P<0.05 were considered significant.

## 3. Results

Our results indicated that administration of 1 and 10 mg/ml of Concentration of juniper communis resulted in significant decrease in viability of Bhk cells compared to control cells (p<0.05 and ). Administration of 0.1, 0.001 and 0.01 mg/ml of of Juniper communis did not significantly change viability of Bhk cells compared to control group.

## 4. Discussion

Many natural products have biological activity that can be of therapeutic benefit in treating diseases. Indeed, during the very last years, after the advent of molecular biology and combinatorial chemistry, natural products have partially lost their edges, assuming a secondary role in drug discovery and drug development. However, more recent times have seen a rejuvenated and renewed interest for natural compounds and their role as powerful basis for drug development [10]. But many of them remain untested and their use are either poorly monitored or not even monitored at all. There is no gainsaying the fact that the requirements as well as the research protocols, standards and methods needed for the evaluation of the safety and efficacy of herbal medicines are much more complex than those required for conventional. A single herbal medicine or medicinal plant may contain hundreds of natural constituents, and a mixed herbal medicinal product may contain several times that number[11].that The need to review them on normal cells are created.Considering that J. communis berries have been observed in a wide range of traditional medicinal systems [5]. this study examines the effect of extracts of juniper on viability cells Bhk deals.

The results of this study indicate that increased concentrations of juniper extract reduced the viability of cell lines Bhk. At low doses, the extract had no effect on cell lines and increase concentration, prevents the proliferation Cells. In fact, the effect is dose dependent. This finding is consistent with the evidence in the study Bnklr and colleagues (2013), which show At higher concentrations two extracts from medicinal plants, i.e., walnut husk extract and spent hop extract, all tested compounds caused significant loss of cell viability [12] Junipers communis is a good natural source for production and pharmaceutical preparation of antibacterial drugs [13]. Due to the wide use juniper extract in the treatment of diseases, according to studies such as antibacterial, anti-inflammatory, [14] Antiseptic genitourinary system Regulate and control urination dysuria[16], and its role as an anti-tumor agent [17] shows the importance of determining the appropriate dose for use of the plant. [15].

Thus the present data indicate the need to establish safe dose for these plant extracts - taking into account the ioavailability factors - as these plants are regularly and commonly consumed. [18]

#### 5. Conclusion

According to our finding, different doses of Juniper have different effects on normal BHK cells viability. In fact, our results indicated that administration of low doses of extract had no effect on cell lines BHK.

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## 7. References

- [1] Mishra A, Seth A, Maurya K. Therapeutic significance and pharmacological activities of antidiarrheal medicinal plants mention in Ayurveda.J Interecult Ethnopharmacol 2016 Apr; 5(3):290-307
- [2] Batugal PA, Kanniah J, Lee SY, JT Oliver.Medicinal planets Research in Asia . International Plant Genetic Resources Institute, 2004.
- [3] Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant.J Adv Pharm Technol Res. 2011 Apr-Jun; 2(2): 104–109. http://dx.doi.org/10.4103/2231-4040.82956
- [4] Asgary S, Naderi Go.A, Shams Ardekani MR, Sandbar A, Airin A, et al. Inhibition of protein glycation by essential oils of branchlets and fruits of Juniperus communis subsp. Hemispheric. Res Pharm Sci. 2014 May-Jun; 9(3): 179– 185.
- [5] Fernandez Al, Cock I.E. The Therapeutic Properties of Juniperus communis L.: Antioxidant Capacity, Bacterial growth Inhibition, Anticancer Activity and Toxicity. Pharmacognosy Journal May 2016;3.17
- [6] Milojeric SZ, Glisic S.B, Skala D.M. The batch fractionation of Juniperus communis L. essential oil: experimental study, mathematical simulation and process economy. Chemical Industry & Chemical Engineering Quarterly 2010;16(2).183-191
- [7] Hernandez R, Brown DT. Growth and maintenance of baby hamster kidney (BHK) cells. Curr Protoc Microbiol. 2010 May;

http://dx.doi.org/10.1002/9780471729259.mca04hs17

- [8] Ahmad R, Sadri M, Pakpour R. The Effects of Hydroalcoholic Extract of Ganoderma lucidum on Breast Cancer Cells in Cell Culture. Available at: iicbe.org.
- [9] Barzgarnejad A, Azadbakht M, Emadian O, Fattahi M. In vitro effect of juniper fruit extract on dissolution of urinary stones. J Mazand Univ Med Sci 2010; 20(75): 31-36 (Persian).
- [10] Pollio A, Zarrelli A, Romanucci V, Mauro A.D, Barr F, Pinto G, et al. Article Polyphenolic Profile and Targeted Bioactivity of Methanolic Extracts from Mediterranean Ethnomedicinal Plants on Human Cancer Cell Lines. Molecules 2016, 21, 395

http://dx.doi.org/10.3390/molecules21040395

[11] Ekor M. The growing use of herbal medicine: issue relating to adverse reactions and challenges in monitoring safety.2014;4(177).

- [12] Boncler M, Różalski M, Krajewska U, Podsędek A, Watala C. Comparison of PrestoBlue and MTT assays of cellular viability in the assessment of anti-proliferative effects of plant extracts on human endothelial cells. Journal of Pharmacological and Toxicological Methods. January–February 2014;(1),9-16 http://dx.doi.org/10.1016/j.vascn.2013.09.003
- [13] Al-Roubaeay D. Extraction of Essential Oil from Juniperus Communis and Study its Effect on the Growth of Bacteria and Yeast isolated from urinary tract infections. Iraqi J. Comm. Med. April. 2006 19 (2)
- [14] Barjaktarevic B, Sovilj M, Knez Z, Agricle. 2005, Food Chem. (53) 2630.
- [15] Evans WC. Pharmacognosy Trease and Evans. 15th edition, London: Elsevier Science, 2002. P 46.
- [16] Ballabh B, Chaurasia OP, Ahmed Z, Singh SB. Traditional medicinal plants of cold desert Ladakh-used against kidney and urinary disorders. J Ethnopharmacol. 2008 Jul 23;118(2):331-9 http://dx.doi.org/10.1016/j.jep.2008.04.022
- [17] Damnjanovic B M. Thesis M Sc, 2000, Faculty of Technology and Metallurgy. Belgrde, (in Serbian)
- [18] Norfaizatul SO, Zetty Akmal CZ, Noralisa AK, Then SM, Wan Zurinah WN, Musalmah M. Dual Effects of Plant Antioxidants on Neuron Cell Viability.march2010; 9 (6)