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The Effects of *Frangula Alnus miller* on HEK Cells in Cell culture

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Abstract: *Rhamnus frangula (synonym Frangula alnus) is a tall deciduous shrub in the family Rhamnaceae. It is native to Europe, northernmost Africa, and western Asia, from Ireland and Great Britain north to 68°N in Scandinavia, east to central Siberia and Xinjiang in western China, and south to northern Morocco, Turkey, and the Alborz and Caucasus Mountains; in the northwest of its range, it is rare and scattered and the studies show relationship between Frangula Alnus and proliferation of stem cells. The main aim of this study was to determine the effects of Frangula Alnus miller extract on HEK Cells in Cell culture. In this laboratory experimental study, HEK cells were exposed to 10,1,0.1,0.01 mg/ml Frangula Alnus in cell culture. After 48 hours, the viability of HEK cells was examined using MTT assay. The data was analyzed using ANOVA. Our findings show that viability of HEK cells decreased significantly when exposed to 10 m/ml of Frangula Alnus. Other doses of Frangula Alnus resulted in increased viability.*

Keywords: *Frangula Aluns , Viability , HEK cell line*

1. Introduction

Rhamnus frangula Linn. (synonym *Frangula alnus Miller*, Rhamnaceae family) (buckthorn) is a shrub to small tree that was introduced in the Midwest in 1849 as an ornamental plant [1]. F. alnus typically inhabits wet terrestrial sites, including poorly drained woodlands, riparian areas, and wetlands [2].It grows rapidly, forms dense thickets, and retains leaves late into fall [1], [3]-[5]. This plant is native to Europe, northernmost Africa, and western Asia, from Ireland and Great Britain north to 68°N in Scandinavia, east to central Siberia and Xinjiang in western China, and south to northern Morocco, Turkey, and the Alborz and Caucasus Mountains; in the northwest of its range (Ireland, Scotland), it is rare and scattered. It is also introduced and naturalised in eastern North America [6]-[10].

Human Embryonic Kidney cells, often referred to as HEK 293, HEK-293, 293 cells . The original 293 cells were derived in 1973 from the kidney of an aborted human embryo of unknown parenthood by transformation with sheared Adenovirus 5 DNA. The HEK293 human cell lineage is widely used in cell biology and biotechnology because their growth are very easy [11].

The results of studies on the leaves of this plant have shown that F. alnus leaf litter has the capacity to alter soil properties and microbial function by stimulating N mineralization [12]-[16]. Bark of *Frangula alnus* Mill also referred as *Frangulae cortex* is widely used as laxative and can be found as component of herbal laxative preparations Laxative property of *F. alnus* bark has been attributed to the presence of anthraquinone glycoside derivatives, glucofrangulins and frangulins [17]. The most represented anthraquinone derivatives in F. rupestris and F. alnus bark were physcion (0.11 mg/g) and emodin (2.03 mg/g), respectively [18]. 1,6,8-trihydroxy-3-methyl-anthraquinone (emodin) exhibits numerous biological activities such affects the immuno-system , repairing the UV-induced DNA damage, acting on vasomotor system, and having anti-inflammatory and analgestic effects[19]. Past studies have shown that Emodin was genotoxic [20]. It has also been shown that emodin is not carcinogenic (there was no evidence of carcinogenic activity in male rats and female mice, and equivocal evidence in female rats and male mice), although pathological changes in renal tubule were observed

[21]. According to previous studies , laxative effect of preparations based on *F. alnus* bark (as well as other anthraquinone-containing laxatives) is exerted by damaging epithelial cells that can lead to development of colorectal carcinoma, only short-term use of such preparations in case of occasional constipation is recommended [22],[23].

In contrast previous study , neither anthranoid laxative use, even in the long term, nor macroscopic or marked microscopic melanosis coli were associated with any significant risk for the development of colorectal adenoma or carcinoma [24]. Besides their purgative properties, anthraquinones possess antifungal [25], [26] , antiviral, antibacterial [18], [27]-[29], anticancer [30] , and antioxidant [18], [31], [32]. Plant phenolics are multifunctional antioxidants which can act as reducing agents, free radical scavengers, metal chelators, and singlet oxygen quenchers thus inhibiting processes that can lead to membrane damage, ageing, heart disease, and cancer [33].

According to previous studies, antioxidant properties of the bark extract were attributed to its high phenolic and flavonoids content [18], [34]. Results of a study on antioxidant activity showed that, unlike emodin, bark extract possess moderate antioxidant capacity (44.6%, 46.8% and 2.25 mmol Fe2+/g measured by DPPH, ABTS and FRAP assay, respectively) that can be related to relatively high phenolic content (116.07 mg/g) [34]. The results of stdies about antioxidant and antimicrobial activity of F. rupestris and F. alnus bark has shown that oth species demonstrated excellent antioxidant and antimicrobial activities [18]. A recent study has analyzed the qualitative (HPTLC) and quantitative (UV/VIS spectrophotometry and HPTLC densitometry) of the main constituents [total hydroxymethylanthraquinones as 1,8-dihydroxyanthraquinone and 1,6,8-trihydroxy-3-methyl-anthraquinone (emodin)] of plant material (R. frangula bark) and frangula bark extracts obtained by different extraction processes and with a different solvent (EtOH, alkaline water) [35].

2. Materials and Methods

In this laboratory experimental study, *Frangula Alnus miller* was prepared as powder and different concentrations of (10,1,0.1,0.01 mg/ml) *Frangula Alnus miller* were used in our study. We used MTT assay in this work to determine the effects of Frangula Alnus miller on HEK cells viability in cell culture. Briefly, the procedure was continued and carried out in the following steps:

Day One: 100µl 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 Frangula Alnus miller was added and incubated at 37 with 5%co2 overnight. For control 10%FBS was added to media.

Day Three: Frangula Alnus miller was removed from media, 20µl 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 570nm with a reference filter of 630 and recorded.

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

3. Results

Figure I shows the viability of HEK cells in response to different doses of Frangula Alnus miller.

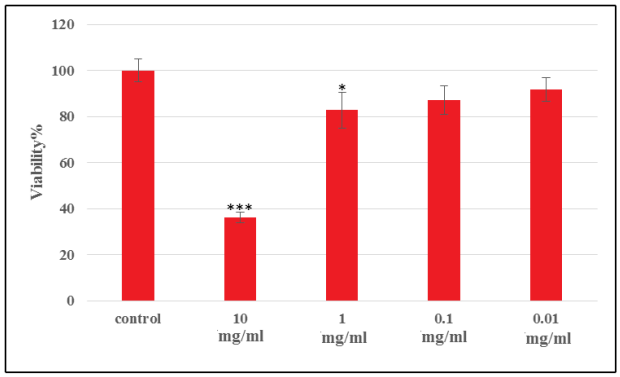

Fig I. Viability of HEK cells in response to different doses of Frangula Alnus miller in cell culture**.**

Our findings show that viability of HEK cells decreased in response to 10 mg/ml of *Frangula Alnus miller*. However, viability of HEK cells did not change in response 0.01, 0.1, 1 mg/ml of *Frangula Alnus miller* extract. * and *** indicate significant difference compared with control group at P <0.01 and P <0.001, respectively .

4. Discussion

Our findings show that high dose of *Frangula Alnus miller* extract has cytotoxic effects on normal kidney cells, however, low doses of the extract did not influence viability of normal kidney cells, according to which, *Frangula Alnus miller* extract when administered in lower concentrations do not impair normal cells.
Since *Frangula Alnus miller* extract possess moderate antioxidant capacity [36], it may have protective effects on healthy cells. *Frangula Alnus* leaf litter has also antimicrobial effects [37]. Since the most represented anthraquinone derivatives in F. alnus bark is emodin [18], which has significant pathological effects on renal tubule [21], it is suggested that the effects of high dose of *Frangula Alnus miller* extract on normal kidney cells may come from emodin cytotoxic effects.

5. Conclusion

Our results showed that high dose of *Frangula Alnus* extract had cytotoxic effects on HEK cell, however, low doses of the extract did not influence viability of normal kidney cells.

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