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Yuliia Haluzinska[1]
Mikhail Baranovsky[2]

APPLICATION OF MISTLETOE IN CANCER TREATMENT

National Aviation University,
1, Kosmonavta Komarova ave., Kyiv, 03580, Ukraine
E-mails: [1]jhaluzinska@ukr.net; [2]izbarvinok@gmail.com

Abstract

Objective: *natural method of cancer treatment with the help of Viscum AlBum is described in the article. Chemical composition, application of mistletoe in medicine and influence on cell lines (NFS-60 and L-41) are considered.* ***Methods:*** *this article considers a microscopic method based on inhibition and quantification of cancer cell lines.* ***Results and discussions:*** *experiment shows that mistletoe dried and fresh extract (from leaves and steams) is effective against both myeloid cancer cells: mouse (NFS-60 suspension) and human (L-41 monolayer). Inhibition of L-41 myeloid cell occurs, because the values of optical density are no more than 1 due to wave length 590 nm. Inhibition of cancer cell occurs, the values of optical density are no more than 0, 1 due to wave length 570nm.*

Keywords: extraction; inhibition of cancer cell lines; L-41(monolayer) cell line; mistletoe; NFS-60(suspension) cell line; *Viscum Album.*

1. Introduction

Technological parameters of plant material are crucial factors in the achievement of the therapeutic effect of phytomedications. It concerns the type of plant material (flowers, leaves, rhizomes, etc.), solvent and ratio of extractant-raw material, degree of milling of raw material, which is a very important technological parameter since it determines the process of mass transfer in extraction and extraction time.

Increased demand for medicines of natural origin stimulate the search for new plants with a certain spectrum of pharmacological action, and to optimize the use and in-depth study of raw materials traditionally used in medicine. One such plant is mistletoe. Mistletoes of the *Loranthaceae* and *Viscaceae* are hemiparasitic plants and their preparations in the form of injectable extracts, infusions, tinctures, fluid extracts or tea bags are widely used in various cultures in almost every continent to treat or manage various health problems including hypertension, inflammatory conditions, irregular menstruations, menopause, epilepsy, arthritis, cancer

Parasitic interactions among organisms are highly diverse and play important roles in all ecosystems (Fig.1). These interactions span all group of organisms, from phages and viruses to bacteria, animals (mainly insects) and fungi to flowering plants. A well-known group of flowering plant parasites are mistletoes. The European mistletoe *Viscum album (Viscaceae*) is a known pathogen, a pharmaceutical plant, and a symbol in mythology [1].

Fig. 1. Hemiparasitic mistletoe

Viscum album is an evergreen hemiparasitic shrub, growing on different woody hosts. *Viscum* is dioecious, insect pollinated and has fleshy fruits that are bird dispersed. Based on host specificity, three host races are distinguished in Europe: *V. album* grows on a wide variety of deciduous trees, *V. a. abietis* is restricted to fir (*Abies spp*.), and *V. a*. austriacum occurs mainly on pine (*Pinus spp.).* A fourth host race, *V. a*. *creticum*, is associated with a sole pine host, Pinus halepensis ssp. brutia, and occurs exclusively on the island of Crete [2].

Nowadays, mistletoes are flowering plants that show some degree of parasitism. Mistletoe types include root-parasitic, terrestrial shrubs, common epiphytic stem parasites (e.g. *Viscum album*) and even endophytic species that produce only flowers and fruits on the surface of the host (e.g. *Viscum minimum*). All mistletoes are shrubs and develop a haustorium to contact the host xylem for water and nutrient uptake. They are classified as hemiparasites but are also called water parasites, partial parasites, aerial parasites or epiparasites. Here the functional term hemiparasite is use[3-4].

Viscum album is a mostly globose perennial evergreen shrub with persistent haustoria in the host. Globe diameter may reach up to 150 cm with diachsial branching pattern first forming a fan and with increasing growth forming a globe. Foliage leaves are opposite, rarely 3 (- 4 - 5) whorled, sessile, obovate-oblong, obtuse, leathery and (yellowish-) green. In general leaf length ranges between (1.3-) 2 - 8 (-10.7) cm, with a minimum width of 0.3 cm and a maximum of 4.3 cm. Foliage leaf internodes are 1 - 9 cm long [5-6]. The length of leaves and internodes increases during the first five years after germination and decreases slowly thereafter. Shape and size of leaves may vary considerably, not only within an individual, but between different individuals of the same host tree or of different host trees [7].

The European mistletoe, *Viscum album L. (Viscaceae*), is widely distributed across Europe. It is a perennial evergreen shrub growing as hemiparasite on woody plant species. *Viscum album* is dioecious and insect pollinated. Female and male flowers are yellowish-green and inconspicuous. The white berries are dispersed by a variety of birds, the most important being the mistle thrush (Turdus viscivorus), fieldfare (Turdus pilaris), waxwing (Bombycilla garrula) and blackcap (Sylvia atricapilla). The birds mainly feed on the berries, but intestinal passage is not necessary for germination [8]. A peculiarity of the mistletoe berry is the mucilaginous substance viscin, which is able to stick strongly onto tree bark. Unusual for a European plant, the berries start ripening from November to December and are dispersed between February and May, mainly when the migratory birds fly northwards.

2. Analysis of the latest research and publications.

Last year was represented near 186 publications with the same topic about application of mistletoe in cancer treatment in PubMed library. That is why this problem is actually global on national level and need more research and discussions.

3. Characteristics of investigated cell lines.

So, for the checking antitumor activity of mistletoe we used cancer cell lines NFS-60(suspension) and L-41 (monolayer) . General characteristics of NFS-60 are represented in the table 1and L-41 is human monolayer cancer cell line [9-10].

Table 1

General characteristics of NFS-60 (suspension) cell line

Organism	Mouse
Tissue	Blood
Morphology	Lymphoblast
Cell type	leukemia, myeloid
Growth properties	suspension
Description	A murine myeloblastic cell line established from leukemic cells obtained after infection of (NFS X DBA/2) F1 adult mice with Cas Br-M murine leukemia virus. NFS-60 cells are dependent on IL3 for maintenance of viability in vitro.

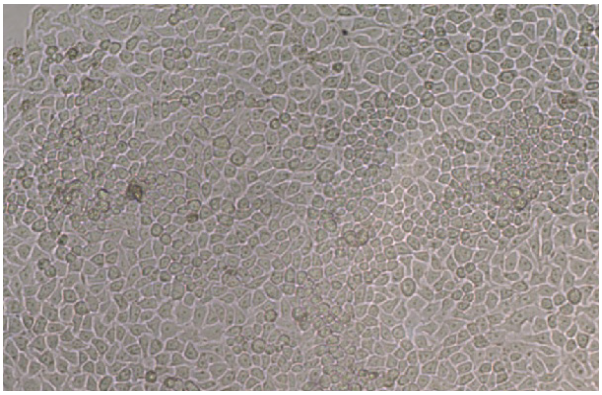

Fig. 2. L-41 (monolayer) cell line under microscope

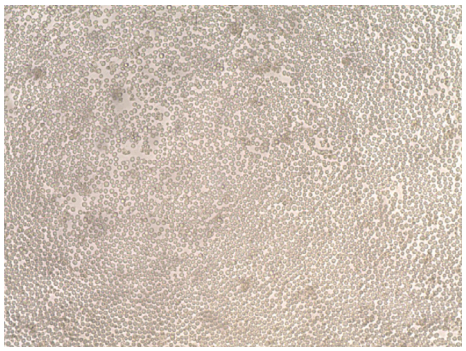

Fig. 3. NFS-60 (suspension) cell line under microscope

Both cell lines were incubated in CO_2-incubator (38^0 C)12 hours with mistletoe extract in different concentrations.

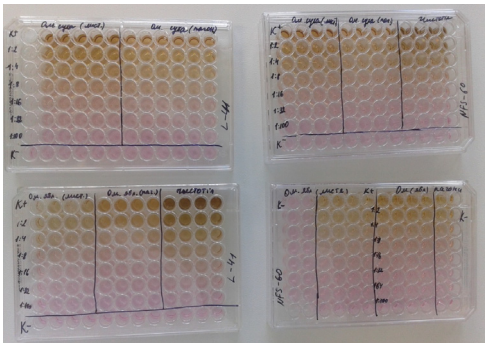

Fig. 4. Cell lines before the incubation.

On the fig. 2 – 3 we observe alive cell lines under normal condition in growth medium without any extracts.

4. Results

The result of investigation shows that tinctures of mistletoe have antitumor activity against NFS-60 (suspension) cell line (Table 2) and L-41 (monolayer) cell line (Table 3).

Table 3

Antitumor activity of mistletoe against NFS-60 (suspension) mouse myeloid cell line

Dilution	Mistletoe from park tree (leave)	Mistletoe from park tree (steam)
K+	0,0475	0,0455
1:2	0,0475	0,0475
1:4	0,0477	0,0467
1:8	0,0455	0,0512
1:16	0,0457	0,0595
1:32	0,0457	0,0637
1:100	0,0675	0,0755
K-	0,1595	0,16425
Dilution	Mistletoe from fruit tree (fresh leave)	Mistletoe from fruit tree (fresh stem)
K+	0,0447	0,0487
1:2	0,0447	0,0457
1:4	0,0475	0,0452
1:8	0,0627	0,0485
1:16	0,0812	0,0545
1:32	0,0865	0,0607
1:100	0,0955	0,0685
K-	0,169	0,191

There are values of optical density with wavelength 570 nm. K+ is optical density values of concentrated extract. B, C, D, E, F, G, are the cell under corresponding dilutions 1:2,1:4, 1:8, 1:16, 1:32, 1:100 of mistletoe extract. K- is optical density values of NFS- 60 cell lines without any extract. If inhibition of cancer cell occurs, the value of optical density will be no more than 0, 1.

Table 4

Antitumor activity of mistletoe against L-41 (monolayer) human leukemia cell

Dilution	Mistletoe from fruit tree (fresh leave)	Mistletoe from fruit tree (fresh stem)
K+	0,1357	0,1562
1:2	0,1247	0,1202
1:4	0,1312	0,1227
1:8	0,1175	0,1070
1:16	0,1120	0,1102
1:32	0,1285	0,1267
1:100	0,1630	0,1260
K-	1,2950	1,2260
Dilution	Mistletoe from park tree (leave)	Mistletoe from park tree (steam)
K+	0,1101	0,0953
1:2	0,1075	0,0851
1:4	0,1041	0,0843
1:8	0,1073	0,0921
1:16	0,1116	0,0911
1:32	0,1191	0,0891
1:100	0,1195	0,1081
K-	1,0566	0,9448

There are values of optical density with wavelength 590 nm. K+ is the holes with concentrated extract. B, C, D, E, F, G, are the cell under corresponding concentrations 1:2,1:4, 1:8, 1:16, 1:32, 1:100 of mistletoe extract. K- is the holes with L-41 cell lines without any extract. If inhibition

of cancer cell occurs, the value of optical density will be no more than 1.

One of the main features of extraction is the choice of raw materials, in this case compared dry and fresh raw materials. The growth of mouse myeloid cells NFS-60 is lowest when using dry mistletoe.

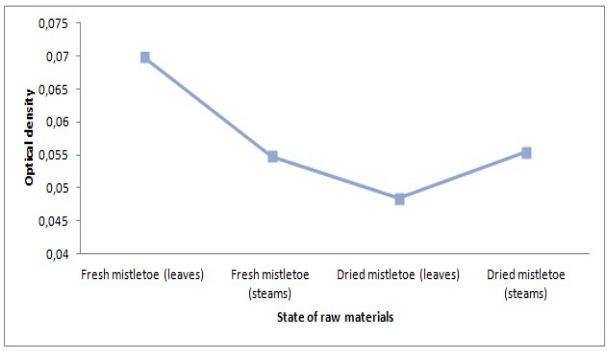

Fig.5. Dependence optical density from state of raw materials.

Content of NFS-60 (suspension) cell line is proportional to the values of optical density. Due to dependence of optical density from dried (Fig. 3.1) and fresh (Fig.3.2) mistletoe extract dilution we observe that inhibition of cancer cell occurs in all dilution but the best results have the less diluted extracts.

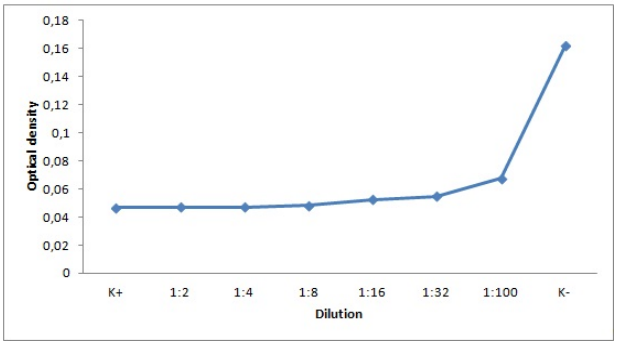

Fig.6. Dependence of optical density from extract dilution of dried mistletoe

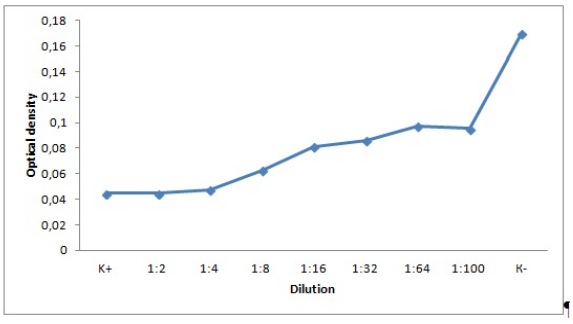

Fig.7. Dependence of optical density from extract dilution of fresh mistletoe

With the help of electronic microscope we also may observe that NFS-60 (suspension) cell line is dead, because all cells are black color and haven`t cell wall (Fig. 8) against the control, where all cells are colored for the best visual perception (Fig.9).

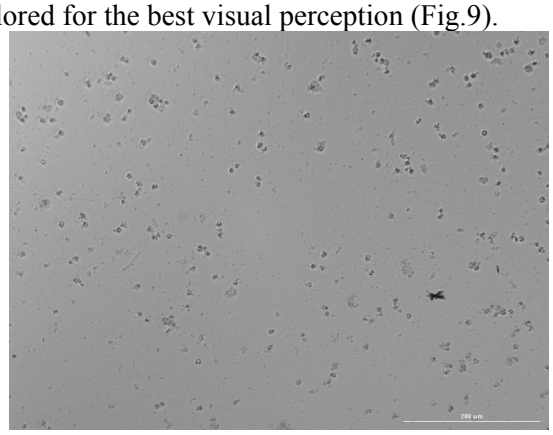

Fig.8. NFS-60 (suspension) under mistletoe fresh extract

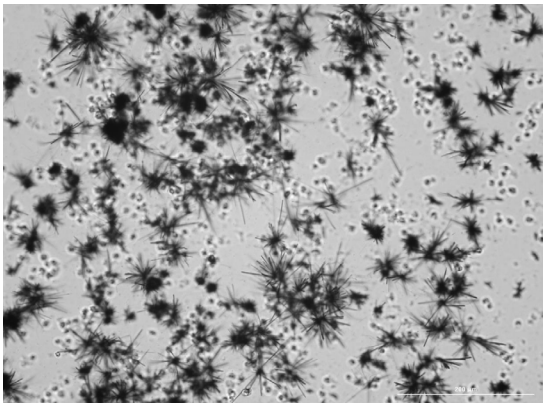

Fig.9. Alive NFS-60cell line (K-)

Content of L-41 (monolayer) cell line is proportional to the values of optical density. Due to dependence of optical density from fresh (Fig. 3.5) and dried (Fig.3.6) mistletoe extract dilution we observe that inhibition of cancer cell occurs in all dilution but the best results have the less diluted extracts.

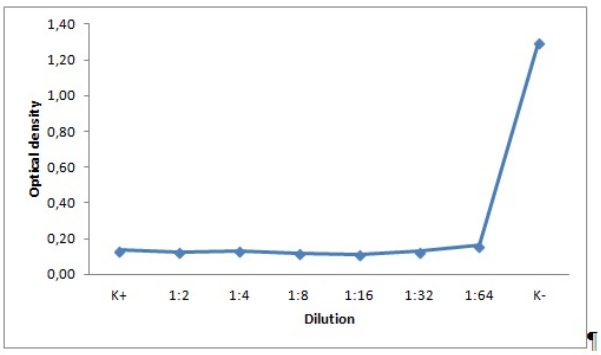

Fig. 10. Dependence of optical density from extract dilution of fresh mistletoe.

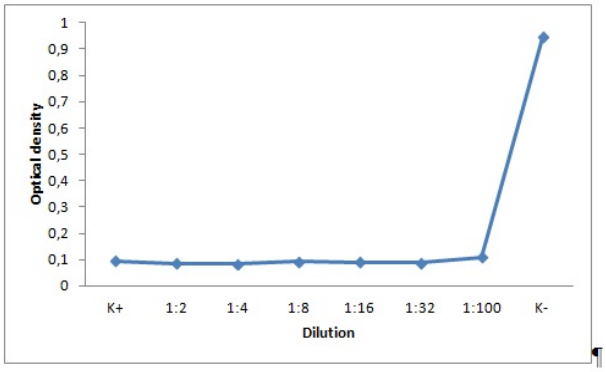

Fig.11. Dependence of optical density from extract dilution of dried mistletoe.

With the help of electronic microscope we also may observe that L-41(monolayer) cell line is dead, because all cells are not in monolayer and there are in disorder (Fig. 12) against the control, where all cells are located on the surface (Fig.13).

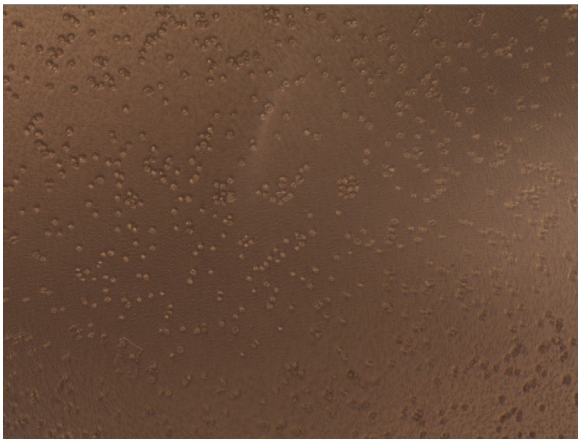

Fig. 12. Dead L-41(monolayer) cell line under mistletoe extract.

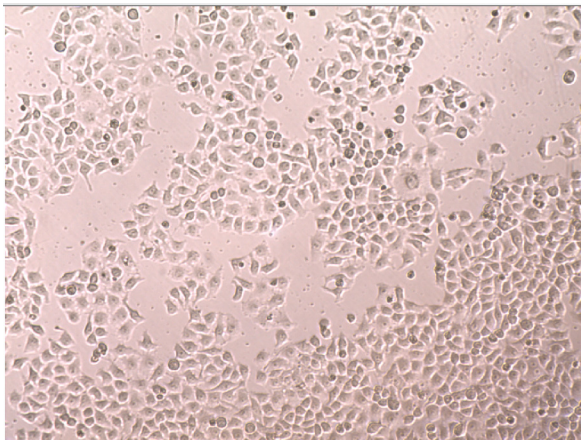

Fig.13. Alive L-41(monolayer) cell line (K-).

5. Conclusions

1. Data processing shows that *Viscum album* is a medicinal herb, with a wide range of pharmacological actions and most effective in cancer diseases.

2. The results of investigation showed that the water extracts of mistletoe possess components that can be used in antitumor drugs for cancer therapy. Experiment shows that mistletoe dried and fresh extract (from leaves and steams) is effective against both myeloid cancer cells: mouse (NFS-60 suspension) and human (L-41 monolayer).

- Inhibition of L-41 myeloid cell occurs, because the values of optical density are no more than 1 due to wave length 590 nm.
- Inhibition of cancer cell occurs, the values of optical density are no more than 0, 1 due to wave length 570nm.

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Ю.І. Галузінська[1]**, М.М. Барановський**[2]

Застосування омели білої у лікуванні ракових захворювань

Національний авіаційний університет, просп. Космонавта Комарова, 1, Київ, 03058, Україна
E-mail: [1]jhaluzinska@ukr.net; [2]izbarvinok@gmail.com

Мета: опис методу лікування ракових захворювань за допомогою Омели Білої. Розглянуто хімічний склад, застосування омели в медицині та її вплив на ракові клітинні лінії NFS-60 (суспензійні), L-41 (моношарові). **Методи:** у статті розглядається мікроскопічний метод, що ґрунтується на інгібуванні та кількісному визначенні ракових клітинних ліній. **Результати та обговорення**: результати дослідження показують, що водні екстракти з омели білої мають такі компоненти у своєму складі, які можуть бути використані при лікування ракових хвороб. Проведений дослід показує, що екстракти із сухої та свіжої сировини (листя та пагони) ефективні проти ракових клітин мієломи миші (NFS-60 суспензійні) та людини (L-41 моношарові). Отримані значення оптичної густини з показниками не більше як 1 за довжиною хвилі 590 нм, свідчать про те що інгібування мієлоїдних клітин людини L-41 відбувається при даних концентраціях. Отримані значення оптичної густини з показниками не більше від 0,1 за довжиною хвилі 570 нм, свідчать про те, що мієлоїдних клітин миші NFS-60 відбувається при даних концентраціях.

Ключові слова: екстракція; інгібування клітинних ліній; Омела Біла; NFS-60 (суспензійні); L-41 (моношарові); *Viscum Album.*

Ю. И. Галузинська[1]**, М.М. Барановський**[2]

Приминение омелы белой в лечении раковых заболеваний

Национальный авиационный университет, просп. Космонавта Комарова, 1, Киев, 03058, Украина
E-mail: [1]jhaluzinska@ukr.net; [2]izbarvinok@gmail.com

Цель: описание метода лечения раковых заболеваний с помощью Омелы Белой. Рассматриваются химический состав, применение омелы в медицине и её влияние на раковые клеточные линии NFS-60 (суспензионные) и L-41 (монослойные). **Методы:** в статье рассматривается микроскопический метод, который основывается на ингибировании и количественном определении раковых клеточных линий. **Результаты и обсуждение:** результаты исследований показывают, что водные экстракты с омелы белой имеют такие компоненты в своем составе, которые могут быть использованы при лечении раковых болезней. Исследования показывают, что экстракты из сухого и свежего сырья (листья и побеги) эффективные против раковых клеток миеломы мыши (NFS-60 суспензионные) и человека (L-41 монослойные). Полученные значения оптической плотности с показателями не более чем 1 при длине волны 590 нм, говорят о том, что ингибирование миеломных клеток человека L-41 происходит при данных концентрациях. Полученные значения оптической плотности с показателями не более чем 0,1 при длине волны 570 нм, говорят о том, что ингибирование миеломных клеток мыши NFS-60 происходит при данных концентрациях.

Ключевые слова: ингибирование клеточных линий; Омела Белая; экстракция; NFS-60 (суспензионные); L-41 (монослойные) клеточные линии; *Viscum Album.*

Yuliia Haluzinska (1994). Master Student.
National Aviation University, Scientific and Research Institute of Environmental Safety, Biotechnology Department, Kyiv, Ukraine.
Education: National Aviation University, Kyiv, Ukraine.
Research area: mistletoe in cancer treatment.
Publications: 2.
E-mail: jhaluzinska@ukr.net

Baranovsky Mikhail (1955). Doctor of Agricultucal Science. Professor.
National Aviation University, Institute of Environmental Safety, Biotechnology Department, Kyiv, Ukraine.
Education: Bila Tserkva Agricultural Institute, USSR (1981).
Research area: mistletoe in cancer treatment.
Publications: 103.
E-mail: izbarvinok@gmail.com.