The International Journal of Business Management and Technology, Volume 8 Issue 4 July-August 2024
ISSN: 2581-3889

Research Article Open Access

The Turkmen Juniper (Juniperus Turcomanica B. Fedtsch) Callus Tissue Lysate' Immunomodulatory Activity

Svetlana Pleskanovskaya[1], Irina Semina[1], Dovlet Dovletov[2]

[1]*Head of Scientific Research Centre, M. Garryyev State Medical University of Turkmenistan, Ashgabat, Turkmenistan,*
[1]*Research assistant Scientific Research Centre, M. Garryyev State Medical University of Turkmenistan, Ashgabat, Turkmenistan*
[2]*Assistant at Department of Medical Microbiology, Virology and Immunology, M. Garryyev State Medical University of Turkmenistan, Ashgabat, Turkmenistan*

Abstract: The attention of the world's scientists is increasingly being attracted not only and not so much by independent medicinal plants, but their callus cells' culture. Callus cells (CC) are essentially stem, totipotent cells; cultures are currently considered as a source and accumulation of tissue parts of any plants and, first of all, economically and biologically significant products. Under natural conditions, callus tissue can appear in plants as a result of mechanical damage. It functions for a short time, protecting the plant in the damaged area and accumulating nutrients for regeneration. In order to obtain a tissue culture, an explant is isolated from any part of the plant (stem, leaf, flower element, root, etc.), sterilized and placed on a nutrient medium of a certain composition.

The aim of this investigation was the *Juniperus turcomanica* (JT) callus cells' lysate (CCL) influence on human and BALB/c mice leukocytes' functional activity *in vitro*. Callus tissue was grown on Murashige–Skoog medium from JT needle explant. CCL was prepared in physiological sodium chloride solution at a ratio of 1:20. The leukocytes' functional activity were determined in modified leukocytes' migration inhibition reaction (LIR). CCL was used as inducer of leukocytes' migration.

It was found that the introduction of JT CCL into the culture medium leads to a significant modulation of the migratory activity of blood leukocytes in both mice and practically healthy individuals *in vitro*. Both the PHP and BALB/c mice' BL respond on JT CCL presence in the culture medium as well by inhibition as stimulation of migration. In PHP, unlike BALB/c mice, in the vast majority of cases, JT CCL *in vitro* inhibits the migration of BL from the glass capillary. In our opinion, JT CCL *in vitro* has a cytokine-like effect on mammalian BL.

Keywords: Juniperus turcomanica, Juniperus turcomanica' callus cells, human and BALB/c mice leukocytes 'migration *in vitro*.

I. Introduction

The attention of the world's scientists is increasingly being attracted not only and not so much by independent medicinal plants, but their callus cells' culture [1, 2, 3]. Callus cells (CC) are essentially stem, totipotent cells; cultures are currently considered as a source and accumulation of tissue parts of any plants and, first of all, economically and biologically significant products [4, 5]. Under natural conditions, callus tissue can appear in plants as a result of mechanical damage. It functions for a short time, protecting the plant in the damaged area and accumulating nutrients for regeneration. In order to obtain a tissue culture, an explant is isolated from any part of the plant (stem, leaf, flower element, root, etc.), sterilized and placed on a nutrient medium of a certain composition. The most important role in inducing callus formation on explants is the presence of specific phytohormones - auxins and cytokines - in the nutrient media [6] .

Currently, CC cultures of cotton, wheat, some medicinal plants have been obtained - ginseng (Ginkgo biloba [7], ammi visnaga, snake rauwolfia (Rauwolfia serpentina), red sparrow (Leptospermum erythrorhizon), valerian (Valeriana officinalis). They are intensively grown and studied in order to obtain fundamentally new herbal medicines. For example, the anti-arrhythmic drug "Aimalin" has been established from the Ajuga reptans and Rauwolfia serpentina

callus cells' biomass. In general, the properties of preparations from callus cells, as a type of herbal preparations, remain poorly studied
[8].
The method of cultivating plants' cells and tissues *in vitro* is of great interest to scientists of Turkmenistan. One of the main areas of research at the Research Center of Turkmen state medical university named after M. Garryyev is phytoimmunoiodulation activity of endemic medicinal plants' callus cells and its lysate. Turkmen juniper - Juniperus turcomanica B.Fedtsch. (MT) is one of the remote junipers produced in the foothills of the South-Western and Central Kopetdag (Turkmenistan) [9]. The healing properties of Turkmen juniper have been studied since the time of Avicenna and have attracted the attention of a large number of modern scientists [10, 11 and 12].
The aim of investigation was Juniperus turcomanica callus cells' lysate influenced determination on human and animals leukocytes' functional activity in vitro.

II. MATERIALS AND METHODS

2.1 Plant material. Callus tissue (CT) was grown in Murashige–Skoog medium [5, 13] from a JT needles explant. The callus cells' Lysate (CCL) was prepared using physiological sodium chloride solution at the ratio of 1:20 [14]. At first the callus tissue was homogenized in a Potter homogenizer, and then the homogenate was frozen and thawed five times. The homogenate was kept for 24 hours at a temperature of +4°C, and then centrifuged at 3000 rpm for 30 minutes. The supernatant was collected in a sterile tube and stored at -20°C until use. All manipulations were carried out in sterile boxing conditions in compliance with aseptic rules.
2.2 Immunological methods. Functional activity of PBL in 30 male BALB/c mice and 75 practically healthy persons (PHP) were studied in a modified reaction of leukocyte migration inhibition (LMIR) [15]. Results are expressed as leukocyte migration index (LMI). [16].
Turkmen juniper (Juniperus turcomanica) (JT) callus tissue lysate (CCL) was used as a migration inducer. When setting up LMIR, 0.3 ml of culture medium and 1.0 µl of LCT were added into the chambers, and then the capillaries with blood were immersed strictly horizontally. After the incubation time, the capillaries were removed and the leukocyte migration index was calculated.
2.3 Statistics. The obtained data were processed using the SPSS program (IBM Corp. 2014. IBM SPSS Statistics for Windows, version 24.0. Armonk, New York: IBM Corp).

III. Research results and discussion

In the course of our work, for almost the first time, we managed to grow callus from explants of Turkmen juniper (Juniperus turcomanica) (photo 1).

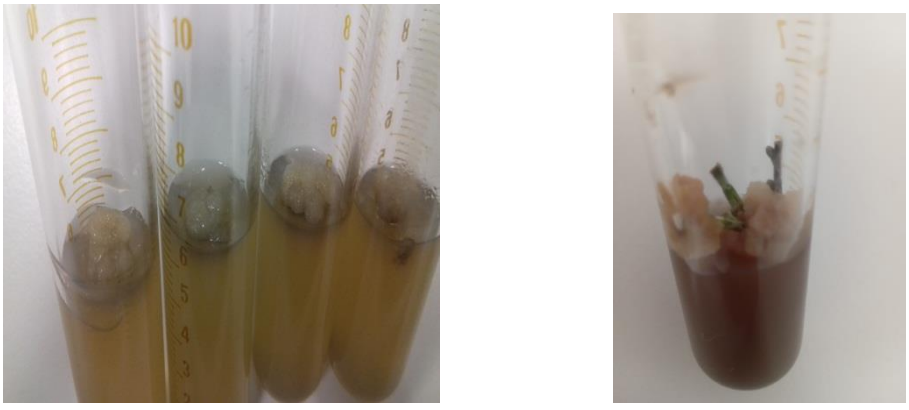
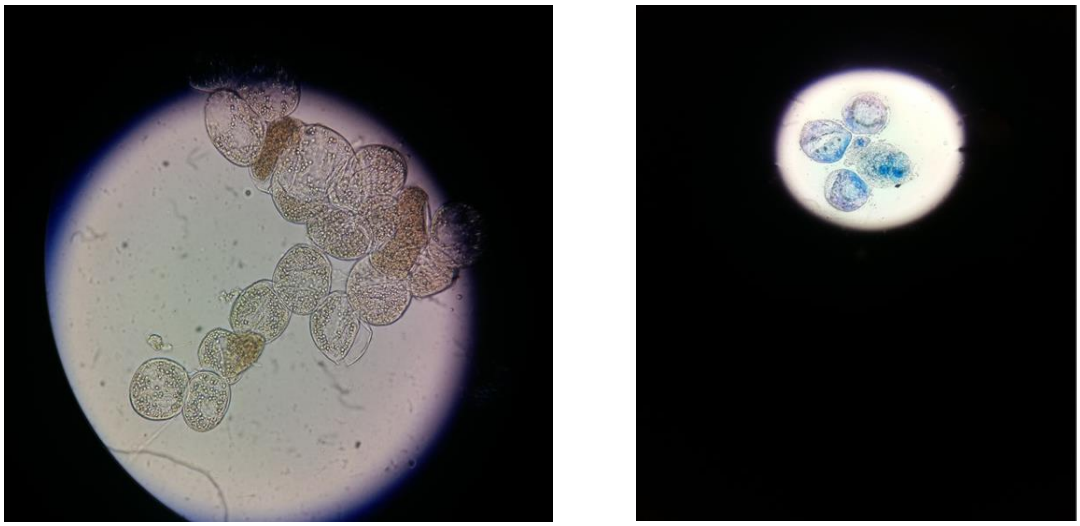


Photo 1. The turkmen juniper (Juniperus turcomanica) callus' tissues

It was this callus tissue that was homogenized. Cells of the JT callus tissue homogenate are shown in the photo. 2.



A B

Photo.2. The juniperus tissue callus cells' homogenate JT: A – native, B – fixed with ethanol and stained with hematoxylin-eosin.

As a result of repeated freezing and thawing, the cells are destroyed (photo 3) and during subsequent centrifugation, their detritus is deposited. The supernatant is used as an inducer of leukocyte migration from a glass capillary in vitro.

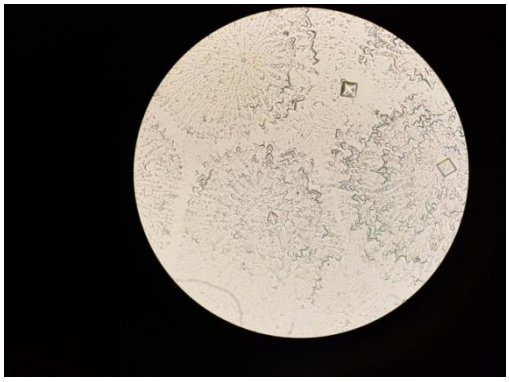


Photo 3. Repeatedly frozen-thawed homogenate of JT callus cells.

It has been shown that the introduction of JT CCL into the culture medium leads to a significant modulation of the migratory activity of leukocytes. Thus, the value of LMI in mice ranges from 67.1 to 112 and averages 69.3 ± 3.5. In the PHP group the LMI ranges from 47 to 130 and averages 84.7 ±9.1 (the difference between the groups is not significant, $p>0.05$).

In the genetically heterogeneous PHP group, the LMI value corresponds to the average value of 64, higher in 20 and lower in 16% of cases (Fig. 1).

Besides, despite the fact that the mice used in the experiments belong to the same genetic line of animals, the LMI *value* corresponds to the average value for the group in 36.6% of animals, exceeds it in 30.1% and is lower in 33.3% of animals. In the group of PHP, the same significant fluctuations in IML values are observed.

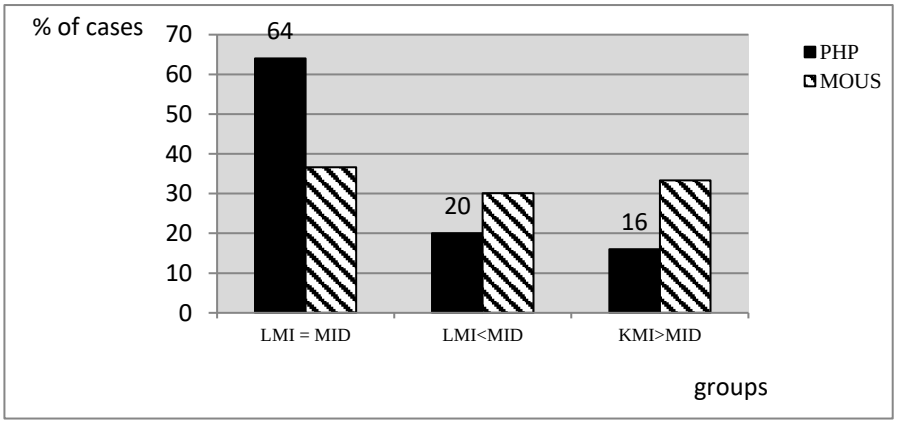


Fig.1. Frequency of correspondence (in %) of the LMI value to the average value for the group in PHP and BALB/c mice.

Thus, leukocytes of both PZL and BALB/c mice in vitro respond to the presence of LCT MTs in the culture medium by inhibiting or stimulating migration. In PZL, unlike BALB/c mice, in the vast majority of cases, LCT MT inhibits the migration of leukocytes from a glass capillary in vitro, that is, it has an anti-inflammatory effect.

V. Conclusion

The attention of the world's scientists is increasingly being attracted not only and not so much by independent medicinal plants, but their callus cells' culture. Callus cells (CC) are essentially stem, totipotent cells; cultures are currently considered as a source and accumulation of tissue parts of any plants and, first of all, economically and biologically significant products. The aim of investigation was *Juniperus turcomanica* callus cells' lysate (JT CCL) influence on human leukocytes' functional activity *in vitro.*

Turkmen juniper - *Juniperus turcomanica B.Fedtsch* is one of the varieties of junipers, grows in the foothills of the South-Western and Central Kopetdag (Turkmenistan). The healing properties of Turkmen juniper have been studied since the time of Avicenna and have attracted the attention of a large number of modern researchers. Relatively recently, it was found that preparations from JT have immunomodulatory properties. In particular, it has been shown that JT decoction *in vitro* modulates the expression of membrane receptors of T-killer cells and NK cells, modulates both spontaneous and tissue antigen-induced migration of leukocytes [10].

Migration is known to be a feature of many cell types, immunocompetent ones especially, in a wide variety of mammalian species [9, 17, and 18]. In this regard, the leukocyte migration inhibition test (LMIR) is used to assess specific cellular immunity.

In the present study, we found that JT CCL *in vitro* modulate the leukocytes' functional activity - both stimulate and inhibit the migration of human and mouse blood leukocytes from a glass capillary.

The ability to inhibit the leukocytes' migration in *vitro* is also characteristic of other plant species' callus cells. For example, callus cells products of Opuntia ficusindica and Prunus avium have the same ability. Based on these data, it was conclude that cherry and cactus callus cells products have an anti-inflammatory effect [19,20].

In our opinion, further study of the Turkmen endemic medicinal plants callus cells' products or its lysates, immunomodulatory activity promising in terms of obtaining new immunomodulatory drugs.

References

[1] Wipa Yaowachai, Prathan Luecha and Worasitikulya Taratima, In vitro callus induction and evaluation of antioxidant activity of Rhinacanthus nasutus (L.) Kurz , *Biol Methods Protoc. 8(1),* 2023; *bpad Published online* 2023 Sep 19. doi: 10.1093/bio methods/bpad019 PMCID: PMC10548163 PMID: 37799729

[2] Sara Alexandra Luis Nunes *Modulation of inflammatory mediators by Opuntia ficus-indica and Prunus avium bio products using an in cell-based model of intestinal inflammation It Dissertation to obtain a Master Degree in Biotechnology September* 2011 https://run.unl.pt/bitstream/10362/6237/1/Nunes_2011

[3] Seyed Mehdi Razavi, Homa Arshneshin and Alireza Ghasemian *In vitro callus induction and isolation of volatile compounds in callus culture of Lallemantia iberica (*M. Biel.) Fisch. & C. A. Mey, *Journal of Plant Process and Function, 18(5),* 2017

[4] Farhad Sheikhnia, Vahid Rashidi, Hossein Maghsoudi, and Maryam Majidinia, *Potential anticancer properties and mechanisms of thymoquinone in colorectal cancer, Cancer Cell Int. 2023, 23{320) Published online* 2023 *Dec 12. doi: 10.1186/s12935-023-03174-4 PMCID: PMC10717210 PMID: 38087345*

[5] *Momoko* Ikeuchi, Keiko Sugimoto, and Akira Iwase1, *Plant Callus: Mechanisms of Induction and Repression OPEN The Plant Cell, 25(31), American Society of Plant Biologists , Japan 2013,*

[6] LIU Xiaozhu, Yoshi Suzuki, Isamu Yamaguchi,. *Tolerance of Ginkgo biloba Callus Cells with Antibiotics[J]. Journal of Southwest Forestry University, 2004, 24(2): 1-2,6. DOI: 10.11929/j.issn.2095-1914.2004.02.001*

[7] Filippova S. N., Volodina S. O., *Anti-inflammatory properties of ekdisteroides in cultures of callus cells of Serrtula coronate and Ajuga reptans, Chemistry of vegetative raw materials, 2002, 1, 57-62.*

[8] Berdimuhamedov G.M., *Medical plants of Turkmenistan (Ashkhabad: Turkmenskaya gosudarstvennaya izdatelskaya sluzba, 2009)*

[9] Pleskanovskaya S.A., Mamedova G., Munir Ozturk, Salih Gucel, Ashyraliyeva M., An Overview of the Ethno botany of Turkmenistan and Use of Juniperus turcomanica in Phytotherapy, *In. Genetic resources, chromosome engineering, and crop improvement (Medical plants),* Ed. Ram J. Singh, CRC Press, Taylor &Francis Group, Bosf Raton-London-New York, 2012.

[10] Pleskanovskaya S.A., Tachmukhammedova A.Kh., Durdyeva O.S., *The effect of Turkmen juniper decoction (Juniperus turcomanica. Fedtch.) on the expression of membrane receptors of some subpopulations of lymphocytes in vitro - Scientific review. Biological Sciences, 3,*2017.

[11] Mobina Alemi, Farzaneh Sabouni, Forough Sanjarian, Kamahldin Haghbeen, *Anti-inflammatory Effect of Seeds and Callus of Nigella sativa L. Extracts on Mix Glial Cells with Regard to Their Thymoquinone Content, AAPS PharmSciTech, 14(1),* 2013

[12] Karomatov I.D., Davlatova M.S. *Juniper in folk and scientific medicine // Electronic scientific journal Biology and Integrative Medicine,1(18),* 2018

[13] Murashige T., Skoog F. A., *Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures, Physiologia Plantarum,15 (3),* 1962.

[14] *Pharmacopoeia of the USSR. Ed. 11. Issue 2. – M.: Medicine.* – 1993.

[15] Frimel G., *Immunological methods. Moscow : Medicine,* 1987.

[16] Pleskanovskaya S.A., *Cellular and humoral immune response in cutaneous leishmaniasis (experimental studies and observations) Abstract. Diss. Cand. Honey. Sci. Moscow-*1992

[17] Khaitov R.M., *Immunomodulatory: myths and reality. Immunologiya.* 2020, *DOI: 10.33029/ 0206-4952-2020-41-2-101-106*

[18] Khaitov, R.M. Yarilin, B.V. Pinegin, *Immunology [Electronic resource]: atlas, GEOTAR-Media* ,2002.

[19] Wu JY1, Feng L, Park HT, Havlioglu N, Wen L, Tang H, Bacon KB, Jiang Hz, Zhang Xc, Rao Y. The neuronal repellent Slit inhibits leukocyte chemotaxis induced by chemotactic factors, Nature, 410(6831), 2001 *Apr 19;:948-52; . 52*

[20] Kenneth M. Murphy, Casey Weaver, Janeway's immunobiology, in 9th edition. New York London: GS, Garland Science, Taylor & Francis Group, 2017. *ISBN 978-0-8153-4551-0, 978-0-8153-4445-2, 978-0-8153-4505-3.*