Black mulberry (Morus Nigra) callus tissue lysate effect on mouse blood leukocytes

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Abstract. The purpose of the research was to study *in vitro* the effect of black mulberry (*Morus nigra*) callus tissue lysate (MNCCL) on the ability of peripheral blood leukocytes of experimental animals to migrate from a glass capillary. The results were expressed as leukocyte migration index (LMI). It was found that *in vitro* in the presence of MNCCL the value of LMI ranges from 63 to 125. That is, both inhibition and stimulation of leukocyte migration are observed. When esophageal tissue antigen (ETAG) is introduced into the incubation medium, the value of LMI also fluctuates from a sharp inhibition of migration to significant stimulation. The aim of the study was to investigate *in vitro* the effect of a black mulberry (Morus nigra) callus cells lysate (CCL) on the ability of peripheral blood leukocytes of experimental animals to migrate from a glass capillary. The effect of CCL on the functional activity of mouse blood leukocytes (MBL) was studied in a leucocytes migration inhibition reaction (LMIR) in a modification of one of the authors of the article. The results were expressed as a leukocyte migration index (LMI). It was found that *in vitro*, CCL significantly affects the migration of MBL from a glass capillary. LMI ranged from 63 to 125. That is, from inhibition to pronounced stimulation of migration. Moreover, when a tissue antigen of the esophagus (ETA) is introduced into the incubation medium, CCL modulates not only the spontaneous but also the tissue antigen-induced migration of leukocytes. In this case, the value of LMI also ranged from a sharp inhibition of migration to significant stimulation. That is, CCL *in vitro* exhibits the properties of both pro- and anti-inflammatory cytokines.

1 Introduction

Medicinal plants were the first remedies to which both animals and humans turned. Turkmenistan is home to 2850 species of higher plants, of which 332 are endemic [1]. This opens up unique opportunities for the wide range of diseases' phytotherapy development. Black mulberry (*Morus nigra*) is an example of one of the most popular medicinal plants in folk medicine.

However, it is not so much the medicinal plants themselves, but rather their callus tissues that are attracting increasing attention from researchers [2,3,4]. Callus cells (CC) are, in essence, stem, totipotent cells, whose cultures are considered as a source for obtaining and accumulating tissues of any plant parts, and primarily those that are

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economically and biologically significant [5,6,7,8]. Callus tissues, as well as preparations from whole plant fragments - leaves, roots, branches, and bark, have anti-inflammatory, antioxidant, and a number of other properties. However, their immunomodulatory activity has been insufficiently studied [7,8].

Leukocytes, the so-called "white blood cells," are the most important function of leukocytes - recognition and phagocytosis, that is, the absorption by a cell of large macromolecular complexes or particles foreign to the organism. Leukocytes, especially neutrophils, are mobile; they are able to migrate along the walls of blood vessels, as well as in the tissues of the body. The ability to migrate is one of the main functional features of leukocytes. A huge number of internal and external factors control the migration of leukocytes, including preparations from plants [9,10,11].

2 Objective

To study the effect of black mulberry (*Morus nigra*) callus tissue lysate on the ability of experimental animals' peripheral blood leukocytes migrate from a glass capillary *in vitro*.

3 Materials and methods

In the experiment, 30 male non-linear white mice weighing at least 20.0 g were used. Mice were obtained from the nursery of the Techno Center of the Academy of Sciences of Turkmenistan. Blood for the study was taken into sterile heparinized glass capillaries from the animals' tail vein.

Callus cells (CT) was grown on Murashige-Skoog medium [13] from *Morus nigra* leaf ' explants. CC lysate (CCL) was prepared on a sodium chloride physiological solution at a ratio of 1:20 [14]. Obtained callus tissue was homogenized in a Potter' homogenizer, then the homogenate five times was frozen and thawed. After the last freezing, the homogenate was incubated at a temperature of +4°C for 24 hours, and then centrifuged at 3000 rpm for 30 minutes, the supernatant was collected in a sterile tube and stored until use at -19°C. All manipulations were carried out in sterile conditions of the box, observing the rules of aseptic technique. The *Black mulberry* callus cells lysate (CCL) was thawed once immediately before the reaction.

The effect of CcL on the functional activity of mouse blood leukocytes (MBL) was studied in the leucocytes migration inhibition reaction (MIR) in a modification [15]. The results were expressed as a leukocyte migration index (LMI). When setting up MIR, 0.01 ml of CTL and/or the esophagus soluble tissue' antigen (ETA) were added to the capillaries cultivation chambers. An equal volume of physiological sodium chloride solution was added to the control chambers.

ETA was prepared from the esophageal tissue of 5 mice by the water-salt extraction method, dosed according to the protein content, which corresponded to 20 mg/ml [16].

The obtained results were mathematically processed using the SPSS for Windows program (USA).

4 Results

It was established that *in vitro* CCL significantly modulate the migration of MBL from a glass capillary. LMI ranged from 63 to 125. The average value of LMI for the group of mice was 82.1±8.3 (Fig.1).

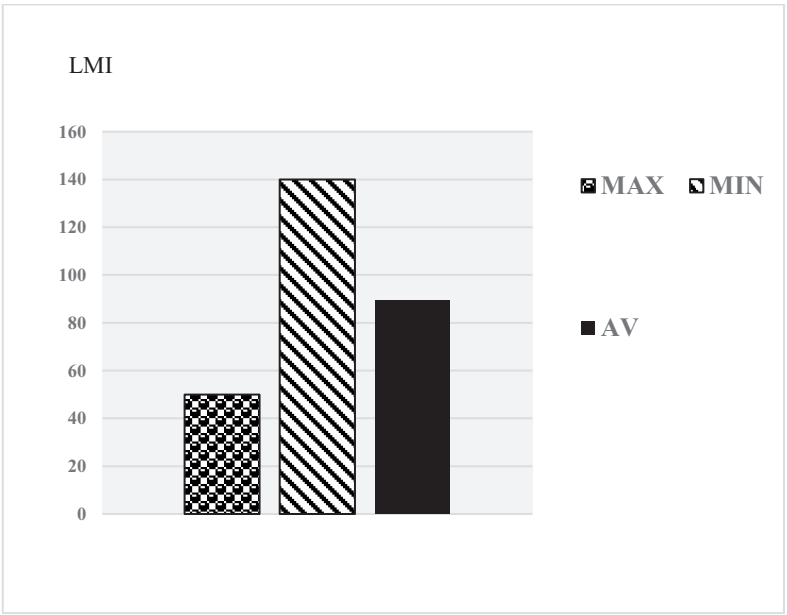

Fig. 1. LMI values in the presence CCL.

That is, the effect ranged from inhibition to pronounced stimulation of leucocytes migration. The diagram (Fig. 2) shows that in 50% of animals, the value of LMI in the presence of CCL exceeds the average values for the group (stimulation of leukocyte migration is observed) and in 30% of cases is significantly reduced compared to them (inhibition of migration). In the remaining cases, the value of LMI corresponds to the average values for the group of animals.

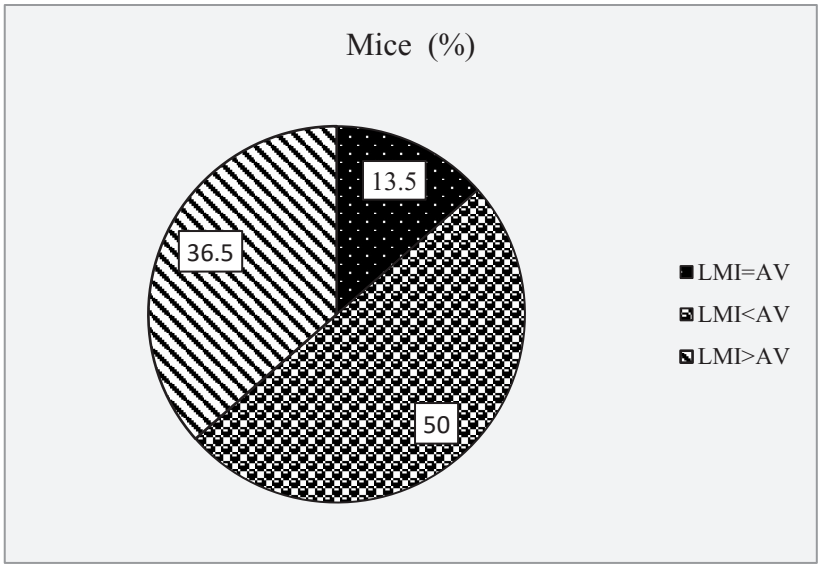

Fig. 2. The IML in the CCL presence value fluctuations

We found it interesting to determine not only the CCL *in vitro* effect on the spontaneous migration of MBL character, but also on the migration of leukocytes induced by the tissue antigen. For the study, we chose the mouse esophageal tissue antigen, as an organ that is one of the first contacted with food agents, including plants. It has been shown that the ETA introducing into the incubation medium, the value of LMI ranges from 50.0 to 130.0 and averaged 79.2 ± 6.5 (Fig. 3). The difference in relation to CCL is not significant ($p > 0.05$).

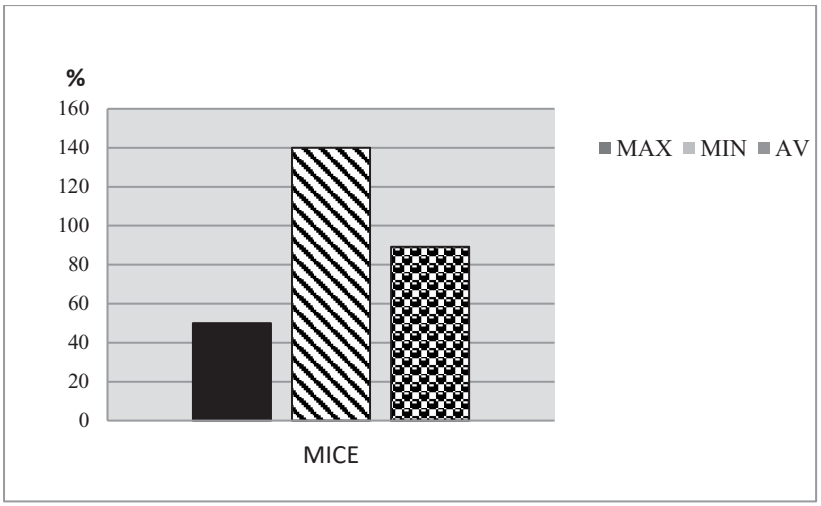

Fig. 3. The IML values in the ETA presence

The LMI value in the ETA presence does not have such pronounced individual fluctuations, which are observed when CCL is introduced into the medium (Fig. 4).

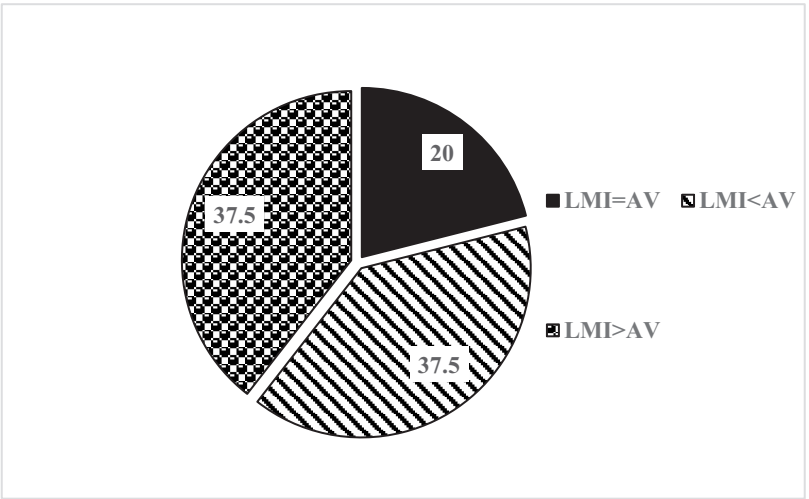

Fig. 4. Frequency of IML value fluctuation in the ETA presence

In almost equal percentages of cases, the value of LMI is either equal, increased or decreased compared to the average values for the group. That is, ETA *in vitro* practically

equally stimulates and inhibits the response of leukocytes to esophageal tissue antigen (fig. 4).

Simultaneous introduction into the capillaries cultivation medium of tissue antigen and lysate of CCL also leads to a LMI values fluctuation from 29 to 125 and averages 80.5 ± 6.8. In this case, the frequency of inhibition and stimulation of migration is almost the same (fig. 5). That is, mctlt in equal percentages both inhibits and stimulates the response of mouse pbl to esophageal tissue antigen."

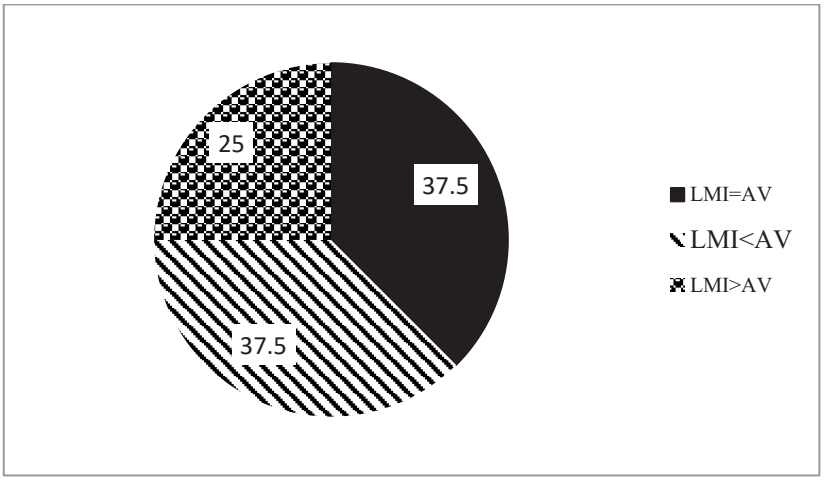

Fig. 5. Frequency of LMI value fluctuation in the CCL and ETF presence

In other words, CCL equally inhibits and stimulates the mouse leukocytes' response to the esophageal tissue antigen

5 Discussion

Migration is a characteristic of many cell types in a wide variety of biological species. The ability of leukocytes to migrate characterizes specific cellular immunity [13,14]. Mobility is a crucial activity of immune cells, allowing them to patrol tissues during differentiation, exchange information, and perform their effector functions. All immune cells exhibit high migratory activity [10,11,18]. A disruption in leukocyte mobility underlies the immunopathogenesis of many, if not all, diseases, since leukocytes, as the primary participants in both innate and adaptive immunity, play a leading role in the development of viral and bacterial infections [15,16]. Neutrophil migration is a key phenomenon in the body's immune response to foreign antigens. The direction and speed of migration are determined by a large number of external factors (also called chemoattractants) and their gradient. Examples of chemoattractants include the 5th component of complement (C5), interleukin 8 (IL-8), N-formyl-methionyl-leucyl-phenylalanine (fMLP), and others [13].

The fact that, *in vitro*, callus cells' lysate (CTL) significantly modulates the leukocytes migration from a glass capillary is of great interest. *In vitro*, CCL functions to regulate leukocyte migration. The magnitude of this migratory index varies significantly. That is, *in vitro* CCL exhibits properties of both pro- and anti-inflammatory cytokines. Moreover, CCL modulates not only spontaneous but also tissue antigen-induced leukocyte migration.

In our view, further research in this direction will not only shed light on the immunomodulatory properties of callus tissues and/or their lysates but also on their ability to influence the cytoskeleton of immune-competent cells *in vitro*.

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