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The influence of Juniperus turcomanica decoction on the healthy persons' peripheral blood leucocytes' content, platelets' number and morphology in vitro

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Abstract: Objective: Juniperus turcomanica: Turkmen juniper (TJ) occurs in the woodland of lower and upper belt of mountains in South-West and Central Kopetdag. The aim of the study was to investigate the effect of TJ 5% decoction on leucocytes' content, platelets' number and morphology in peripheral blood' samplesof the practically healthy persons (PHP) *in vitro* deepens on the duration of the incubation.
Methods: 200 hemograms of PHP had been received on hemoanalyzer ABX Pentra 60 C+ (France). The percentage of white blood cells (WBC), lymphocytes (LYM), neutrophils (NEU), basophils (BAS), eosinophil (EOS), monocytes (MON), the absolute number of atypical lymphocytes (ALY) and large immature cells (LIC), as well as the total number of platelets (RLT, 10^3/mm^3) and mean of platelet volume (MPV, fl) were determined. After the primary measurement the TJ decoction was added into the test tubes with the blood samples, then they were thoroughly mixed on a shaker at 150 oscillations per minute for 5, 30 and 60 minutes at room temperature (+25°C). Then the same parameters were remeasured.
Results: It has been shown that incubation stabilized blood of PHP for one hour at room temperature in a continually stirring is accompanied by changes in leucocytes number and platelets volume, which can consider as result of mechanical stress. Decoction of *Juniperus turcomanicain vitro* increases the resistance of blood leukocytes of PHP to mechanical stress. The obtained data allowto assume that on the plasma membrane of healthy person's platelets and leucocytes' there are receptors that recognize certain components of TJ decoction. These components modulate the functional activity of platelets and atypical lymphocytes.
Keywords: medicinal plants, *Juniperus turcomanica*, human leucocytes, atypical lymphocytes, large immature cells, platelets

Introduction

Juniperus turcomanica B. Fedtsch. Such name was given to one of the varieties of junipers growing in Turkmenistan. *Juniperus turcomanica* - Turkmen juniper (TJ) occurs in the woodland of lower and upper belt of mountains in South-West and Central Kopetdag [18, 19]. In folk medicine, TJ is used as a diuretic, disinfectant, expectorant and means of digestion improvement [4]. The medicinal properties of TJ have been studied since the times of Avicenna [18, 25]. Over the years, TJ has beet attracts the attention of a large number of scientists [1, 12, 18, 19], as well as the authors of this work [9, 10, 23, 24].

The aim of the study wasto investigate effect of the TJ 5% decoction on leucocytes' content, platelets' number and morphology at the practically healthy persons (PHP) peripheral blood' samplesdepending on the duration of the incubation time *in vitro*.

Materials and methods

Object

200 hemograms of PHP aged 22.7 ± 0.9 years had been received on hemoanalyzer ABX Pentra 60 C+ (France). For this purple the 5.0 ml of a blood was taken away from PHP vein with the help of vacutainers (BD VACUTA-INER K2E (EDTA) 5.0 ml). The blood sample was placed intothe especiallydisposable test tubes with EDTA. The percentage of white blood cells (WBC), lymphocytes (LYM), neutrophils (NEU), basophils (BAS), eosinophil (EOS), monocytes (MON), the absolute number of atypical lymphocytes (ALY) and large immature cells (LIC), as well as the total number of platelets (RLT, 10^3/mm^3) and mean of platelet volume (MPV, fl) were determined in blood samples. After the primary measurement the 0.01 ml of 5% TJ decoction was added into the test tubes with blood samples, then it was thoroughly mixed on a shaker at 150 oscillations per minute for 5, 30 and 60 minutes at room temperature (+25°C). Then the same parameters of the blood samples were remeasured.

Herb

Juniperus turcomanica- Turkmen juniper (TJ) for the study were obtained at State Institute of biology and medicinal plants of the Academy of Science of Turkmenistan, in the form of dried chopped pine needles, packaged in paper bags of 50 grams. 5% TJ decoction (infusum ex 10:200) was prepared in accordance with requirements of the Pharmacopoeia (1991) [26] according to the recipe for ***Juniperus communis L.***[22]. The decoction was prepared just before the experiment.

The obtained data were mathematically processed with the help of the SPSS computer program.

Results: Significantly increasing of the MON' percentage and MPV, but reducing of the ALY' number been shown during the first 5 minutes of blood samples incubation without the TJ (P< .05 in all cases). Although, some tendencyto decrease of leukocyte subpopulations' content is remained (Table 1). As can be seen from the table, the difference in all cases mathematically is not significant (P> .05).

Table 1

After 30 minutes of incubation EOS'and the BAS' content rather sharply increases (to 2.1 and 1.7 times, respectively), the MPV continues to increase, but the number of ALY (P < .01) and LIC (P < .05) progressively decreases.

After 60 minutes ofincubation the NEU, MON, BAS' percentage content as well as ALY and LIC absolutenumber and the MPV was significantly reduced (P < .05)in the blood samples. So, the primary parameters of the "white" blood change significantly with increasing of blood samples' incubation time in the conditions of long stirring at room temperature.

Thus, the BAS, ALY, LIC and platelets are the most sensitive to such conditions of cultivation.

We paid special attention to the small subpopulation of leucocytes, such as the LIC (large immature cells) and ALY (atypical lymphocytes). Their identification and counting become possible on the latest generation of hemoanalyzers [6, 7, 15, 16, 21]. Determinations of the LIC and ALY number currently are recommended to use in clinical practice. So, definition of the LIC is recommend for monitoring of patients with myeloid leukemia, [8,11], while ALY - for patients with lymphoid leukemia [3].

Table 2

The chart (Fig.1) reflects the dynamics of atypical lymphocytes' number depending on the incubation time duration. The relative number of ALY and LIC have almost the same exponential trend line. Hence, their relative content during prolonged incubation *in vitro* changes equally. The changes of trend lines' of the absolute number of atypical lymphocytes are the same (Table 1, Figure 1).

It has been established, that in the peripheral blood of the healthy persons aged 19-25 years, living in our hot region, circulates 1.24 ± 0.01% of ALY and 1.0 ± 0.09% ofLIC.Our data corresponds to the literature data [11]. However the number of this lymphocytes' type in process of blood incubation without a TJ progressively decreases (Fig. 1).

Changes of leukogram in the case of TJ addition are presented in the table (Table 2).In the presence of TJ there is decrease of the percent of BAS and NEU but the increase of EOS' percent relative to the initial level. The LYM' content is not changed, while BAS' increases progressively. That is the "sign" of the relative content of the small subpopulations of leucocytes (EOS, BAS) in the blood samples is modified on the opposite in the TJ decoction presence . (Figure 3).Another feature is that the number of ALY in the presence of TJ significantly decreases since the 30-th minute of incubation on 64%, while without TJ - decreases progressively since the 5-th minute of incubation, but only on 28% (Figure 2) . The number of LIC changes wavy. Though, the trend line on the graph indicates the absence of a tendency to change.

In the same time, within the first 5 minutes of blood samples incubationin the TJ presence the MPV increases at about 38.8%. Then it slightly decreases, but remains authentically lowered in comparison with primary values. That is, when blood samples incubated without TJ the MPV is increased gradually in 1.14 times. In the TJ presence - already since the 5-th minute of incubation MPV increases in 1.4 times in comparison with a reference value.

In other words, when blood samples incubated without TJplatelets "swell" gradually. In the presence of TJ – platelets "swell" rapidly and to a greater extent. Thus, *in vitro* within the first 5 minutes of incubation at room temperature the EOS, BAS and platelets react to the addition in the blood sample TJ decoction but not monocytes –in spite of the fact that they are circulating phagocytes. As a real change of the cells' number, that is determined by hemoanalyzer in a closed system (*in vitro*), is impossible, it can be connected with changes of cells' size [15,16].

So, in conditions of stress (blood is for a long time stirred up in a sealed tube at room temperature) at the first 5 minutes the number of MON, ALY and LYK significantly increases.

Consequently, these cells are the most sensitive to stress. The TJ at adding to the culture medium makes leukocytes more resistant to stress. Hence, small subpopulations of white blood cells, like ALY and LYC, deserve close attention as they, in our opinion, may be used as indicators (biological markers) of the medicinal plants' ability to protect blood cells from stress.

Besides, the population of platelets deserves attentionalso. It is known that due to the specific receptors that are present on their surface - TLRs (Toll-like receptors) platelets immediately react to any bacterial antigen, therefore in English literature they were called "roving guards» (noma dicsentinels) [17]. Due to the presence of the open canalicular system (OCS) in platelets the blood plasma and other environment components can get into them [13, 14, 20]. Consequently, not only the antigens but also components of the TJ decoction can get through OCS into the platelets and cause their "swelling". The size of platelet is the trigger of megakaryocytes' activation [2, 5, 27].Most likely, the using of TJ *in vivo*, in our opinion, should lead to the appearance and accumulation of some its components in the blood. As a result, *in vivo*, as well as *in vitro* "swelling" of platelets (enlarged of their size) may occur, what can activate megakaryocytic sprout of hemopoesis and to stimulate thrombopoiesis.

The obtained data allow assuming that on the cytoplasmic membrane of the healthy person's blood platelets and leucocytes there are receptors which discern certain components of TJ decoction. Probably these componentsthrough the expression of membrane receptors modulate the functional activity of platelets and atypical lymphocytes. In our opinion further investigations in this direction will allow to understand the mechanisms of interaction of *Juniperus turcomanica* (and not only of it) with the human organisms as a whole, and with its immune and hemopoietic systems, in particular.

They will provide new insights into the specific roles of the several of blood cells subpopulations in this interaction, from a different point of view to discern the issues of phytotherapy andphytoimmunomodulation.They will allow to receive new representations about a concrete role of various subpopulations of blood cells in this interaction, and also to consider the questions of phytherapy (therapy of diseases by means of medicinal plants) and hytoimmunomodulation (abilities of grasses to modulate the functional activity of immune system) under another aspect sight .

Conclusion

The obtained data allow to assume that on the plasma membrane of healthy person's platelets and leucocytes' there are receptors that recognize certain components of TJ decoction. These components modulate the functional activity of platelets and atypical lymphocytes. These components modulate the functional activity of platelets and atypical lymphocytes. In our opinion further investigation in this direction will allow to decode the mechanism of *Juniperus turcomanica* (and not only it') interaction with the human organism as a whole, and with its immune and hemopoietic systems, in particular.

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References

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[1]. Adams RP. *Juniperus deltoides*, a new species and nomenclatural notes on *Juniperus policarpos*and*Juniperus turcomanica* (Cupressaceae).Phytologia. 2004; 86:49 –53.
[2]. Andrews RK, Berndt MC Platelet physiology and thrombosis. Thrombosis Res. 2004;114: 447–453.
[3]. BelingaSC, Manga J, Mouliom A. at al. Proportion of Non-Iron Deficiency in full blood cell counts with microcytosis in Cameroon.ISLH.2010. Brighton, UK.
[4]. Berdimuhamedov G.M. Medical plants of Turkmenistan. Vol. I. Ed.Ashkhabad: Turkmen State publishing service. 2009. –.. – 385 p.
[5]. Butkiewicz AM,Kemona H, Dymicka-Piekarska VD at al. Platelet count, mean platelet volume and thrombocytopoietic indices in healthy women and men. Thrombosis Research.2006.118(V2):199–204.
[6]. Buttarello M, Plebani M. Automated blood cell counts state of the art. Am. J. Clin. Pathol. 2008;130:104–116.
[7]. Hoffmann J. J. M. L. and Hoedemakers R. M. J. (2004).Diagnostic performance of the variant lymphocyte flag of the Abbott Cell-Dyn 4000 haematologyanalyser.Clinical & Laboratory HaematologyVolume 26, Issue 1, pages 9–13, February (Article first published online: 23 JAN 2004http://onlinelibrary.wiley.com/doi/10.1111/j.0141-9854.2003.00581.x/abstract).

[8]. María E Arroyo, María DTabernero, María AGarcía-Marcos and Alberto Orfao Analytic Performance of the PENTRA 80 Automated Blood Cell Analyzer for the Evaluation of Normal and Pathologic WBCs http://ajcp.ascpjournals.org/content/ 123/2/206.full.pdf
[9]. Pleskanovskaya SA, Gurbandurdiyev A. et all On the possibility of Juniperus turcomanica decoction in the chronic tonsillitis patients' treatment using. Turkmen health care J.2003;4:18 –-20.
[10]. Pleskanovskaya SA, Mamedova G., Munir Ozturk at al. AnOverviev of the Ethnobotany of Turkmenistan and Use of *Juniperusturcomanica*in Phytotherapy. In: Genetic resources, chromosome engineering, and crop improvement (Medicinal plants). Ed. Ram J. Singh, CRC Press, Taylor &Francis Group, Bocf Raton-London-New York. 2012; 6 (8).pp.207–220.
[11]. Portal J, Blanco F,CasanoLat al. Detection of nucleated red cells in peripheral blood samples from pregnant women using ABX Pentra DX 120 analyzer. 2010. ISLH. Brighton, UK.
[12]. Ram J. Singh Landmark Research in medical plants Phytotherapy. In: Genetic resources, chromosome engineering, and crop improvement (Medicinal plants). Ed. Ram J. Singh. CRC Press, Taylor&Francis Group, Bocf Raton-London-New York. 2012; 6 (1) pp.1–13.
[13]. Vasiliev SA, Vinogradov VL, Karabudagova ZK Structure and function of platelets. Gematol. transfuziol.2010; 5: 4–10.(Russian).
[14]. Gavrilov OK, Fainstein FE Depression of hematopoiesis. The Hematology Manual (ed. Vorobyev AI).ed.M: Medicine.1985. (Russian).
[15]. Hematology Analyzers. Interpretation of blood tests (Methodical recommendations). Moscow. 2007:1–2050.(Russian).
[16]. GrachevVV Automatization of laboratory studies. Pentra DX 120. Several aspects of automated clinical diagnostic studies as parameters in hematology. J. "Laboratory" 2013; 3 http: // www. labix.ru/publication/38-article2.html(Russian).
[17]. Danilov IP. Platelets: a new look at their role in the body. Medical news.2008; 9:17–19.(Russian).
[18]. Karryev MO.Archa–medicinal plant. ed.Science:Ashgabat. 1971.(Russian).
[19]. KarryevMO, Artemeva MV,Baeva RT at al. Pharmacology ofmedicinal plants of Turkmenistan (under ed. of Prokopenko AP). Ed.Ashgabat Science.1991.(Russian).
[20]. Korobov FV. Computer morphometric platelet of peripheral blood of healthy persons. Abstract. cand. biol. scienc.Moscow.2001. (Russian).
[21]. Kurilyak OA. The clinical interpretation of some indicators of hematology analyzers 26.05.2009. http://unimedao.ru/articles/-6826/9671-/item/90.(Russian).
[22]. Mashkovskyi MD. Medical products. (V.1). Ed. Moscow: Medicine.1973. (Russian).
[23]. Akyev A., Pleskanovskaya SA. The immune response to *Ekhinococcusgranulosus*cysts' antigen modulation in the *Juniperus turcomanica* decoction presence. Allergology and Immunology.2007; 8(1):104.(Russian).
[24]. Pleskanovskaya SA, Tachmukhammedova AH. Influence of *Juniperus turcomanica*decoction on red blood cells of healthy persons. J.Young scientist.2014;3:87–93.(Russian).
[25]. Reference medicinal plants of Turkmenistan (ed. Karryyev MO). Ed.Ashgabat. Academy of science of Turkmenistan."Encyclopedia".1992. (Russian).
[26]. SSSRM Pharmacopoeia. Ed. 11. Issue 2. Moscow: Medicine.1990.
[27]. Shapiro Amy D. Disorders of platelet function (Amy D. Shapiro Platelet Function Disorders Treatment of Hemophilia - World Federation of Hemophilia1999): Webadmin on May 21, 2003. (Russian)

Drawings to article"The influence of Juniperus turcomanica decoction on the healthy persons' peripheral blood leucocytes' content, platelets' number and morphology in vitro" Svetlana Pleskanovskaya, Aybolek Tachmukhammedova

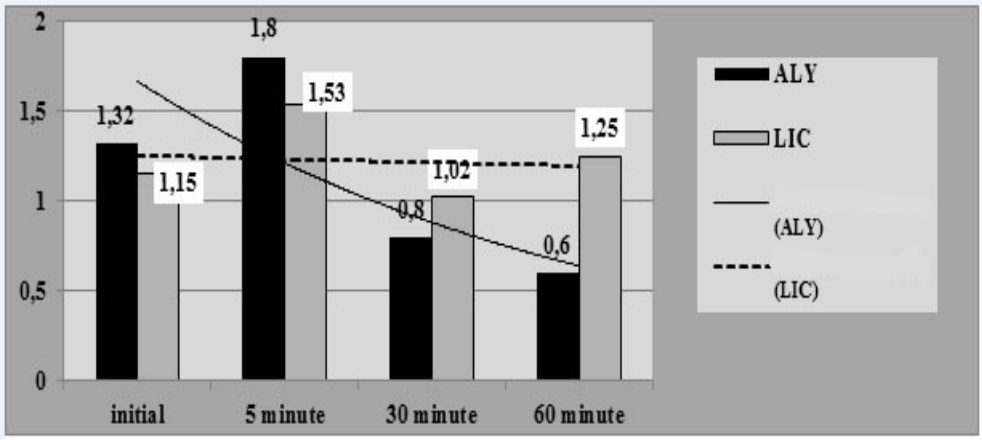


Fig. 1. The atypical lymphocytes (ALY) and large immature cells (LIC)' number in the healthy person's blood samples, depending on the incubation time (without TJ).

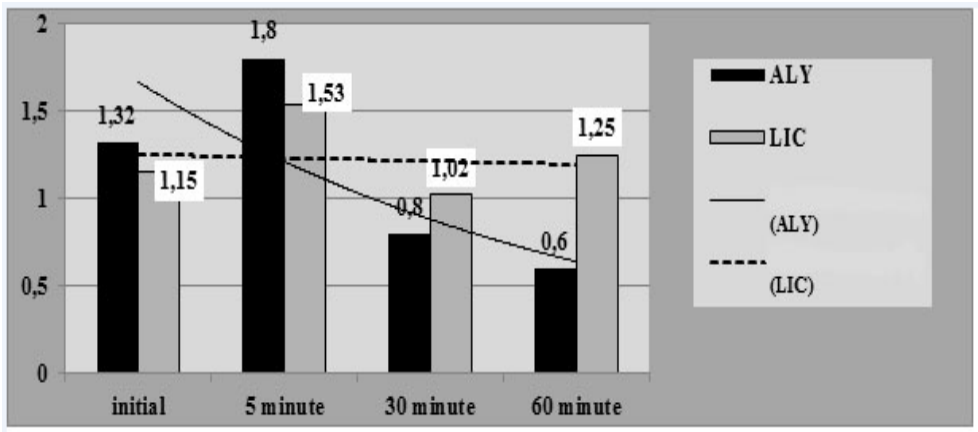
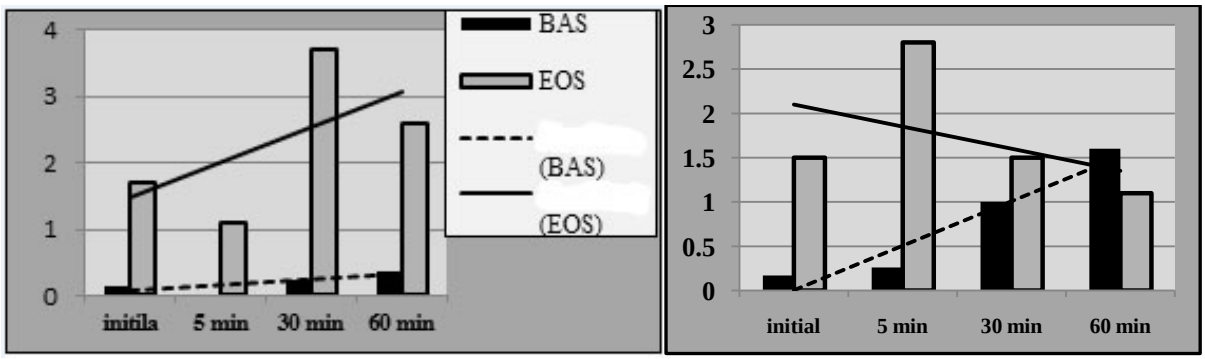


Fig. 2. The atypical lymphocytes (ALY) and large immature cells (LIC)' number in the PHP' blood samples, depending on the time of incubation with TJ.



AB

Fig. 3. Dynamic of the EOS and BAS' number in blood samples during the incubation without of the TJ (A) or with presence of it.

Tables

to article "The influence of Juniperus turcomanica decoction on the healthy persons' peripheral blood leucocytes' content, platelets' number and morphology in vitro" Svetlana Pleskanovskaya, Aybolek Tachmukhammedova

Table 1

Blood indicators of PHP in the dynamics of incubation without the TJ decoction' present

№	Hhemograms indicators	primary data	After 5 minutes of incubation	After 30 minutes of incubation	After 60 minutes of incubation
	WBC(10^3/mm^3)	7,9±3,4	9,2±1,7	7,9±1,0	7,9±1,1
2.	NEU (%)	54,5±4,0	61,8±4,0	54,9±6,3	48,9±3,1*
3.	LYM (%)	28,9±6,7	22,3±2,7	28,75±6,4	29,3 ±4,9
4.	MON (%)	11,2±1,5	14,8±1,7*	11,9±2,5	8,9±1,1*
5.	EOS (%)	1,7±0,9	1,1±0,9	3,7±0,7*	2,6±0,7
6.	BAS (%)	0,15±0,05	0	0,25±0,05*	0,35±0,07*
7.	PLT (10^3/mm^3)	290,0±50	277,0±47	279,5±48,5	270±45
8.	MPV (fl)	7,0±0,23	7,65±0,15*	7,95±0,25*	8,05±0,15*
9.	ALY (absol.number/mm^3)	98,5±6,5	76,5±2,4*	69,0±4,7**	71,3±6, 5*
10.	LIC (absol. number/mm^3)	79,0±6,5	69,5±4,5	55,5±7,3*	49,7±5,1*

Note: * – P < .05; ** – P < .01

Table 2

Blood indicators of PHP in the dynamics of incubation with the TJ decoction

№	Hhemograms indicators	primary data	After 5 minutes of incubation	After 30 minutes of incubation	After 60 minutes of incubation
	WBC (10^3/mm^3)	6,55+0,4	5,2+0,46*	5,45+1,25	5,5+1,1
2.	NEU (**%**)	58+2,0	53,1+6,5	53+1,0*	52+1,0*
3.	LYM (**%**)	31,2+0,3	37,3+3,3*	36+2,5*	36+1,0*
4.	MON (**%**)	9,6+0,3	7,9+0,7*	7,7+0,6	7,15+0,35
5.	EOS (**%**)	1,5+0,1	2,85+0,9*	1,5+0,35	1,1+0,1
6.	BAS (**%**)	0,17+0,2	0,26+0,1**	1,0+0,1*	1.6+0,4**
7.	PLT (**10^3/mm^3**)	228+31	157,3+22,6*	227+55	193,5+41,5
8.	MPV (**μm^3**)	6,6+0,4	9,16+0,76*	7.65+0,25*	7,65+0,25*
9.	ALY (absol.number/mm3)	96,0+23	99,3+28	44,5+13,5*	34+2,0*
10.	LIC (absol.number/mm3)	74,5+9,5	80,6+9,6	56+8,0*	69+8,0

Note: * – P < .05; ** – P < .01