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The geometrid moths (*Lepidoptera, Geometridae, Ennominae*) collected in the Azerbaijan part of the Lesser Caucasus and Middle Araz regions

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This article describes the distribution of 38 species of 26 genera of ~~genus~~ the Ennominae subfamily of the Geometridae family from the Lesser Caucasus and Middle Araz Region and their distribution around the world. Two species of the genera Semiothisa and Dysia are dominant. 16 species were collected from both the Lesser Caucasus and the Middle Araz regions, 10 species only from the Middle Araz region, and 15 species only from the Lesser Caucasus region. According to our information, at least in the middle Araz region, 5 genera and 8 species (*N.divergaria, N.waltheri, S.latirata, D.sicanaria, D.crassipunctata, P.strigillaria, S.sosiaria, S.aesthimata*) are found, there are 12 genera and 16 species in the Lesser Caucasus part of Azerbaijan and 12 genera and 14 species in both regions. One species (*B.betularis* Linnaus, 1758) is new to Azerbaijan and the Lesser Caucasus part of Azerbaijan.

Keywords: *Azerbaijan, Geometridae, geometrid moths, distribution, genus, family, species, GNM*

INTRODUCTION

Geometrid butterflies live in many different biotopes and play a key role in biocenoses and agrocenoses. In the years of mass reproduction, they also cause serious damage to pastures and forest trees. Many of these butterflies are of great economic importance because they are involved in the pollination of flowering plants. The text is based on collection and literature information.

The geometer moths compose one of the largest families of the Lepidoptera including 23000 species from 2000 genera (Didmanidze, 2016). Geometridae is the second largest group of Lepidoptera. Because of their great economic importance, we decided to focus on the history of their studies. The study of geometrid moths in Azerbaijan started in the 19th century. In the second volume of the work of H.Romanov, A.Zeitz, Q.Xristoff, K.Lampert, etc., a large number of butterflies collected from the Caucasus (970 species) were mentioned, 105 species and 2 subspecies of which were geometrid moths. The list of the works of scientists who studied this group until the end of the twentieth century is presented in the references. A.Piriyev systhematically studied the geometrid moths of Absheron (127 species) for the first time. S.Hajiyeva identified 162 species for Mountainous Shirvan, of which 10 species are new to the fauna of Azerbaijan.

In Azerbaijan, this family was studied in the Absheron-Gobustan region only by A.Piriyev (1989), and in the Montier Shirvan region only by S.Hajiyeva (2019).

The Azerbaijan part of the Lesser Caucasus and Middle Araz province includes the districts below: Nakhchivan AR, Ganja, Gazakh, Tovuz, Aghstafa, Gadabay, Kalbajar, Fizuli, Lachin, Zangilan, Aghdam, and Shusha.

The Ennominae subfamily of the Geometridae collection of the Institute of Zoology of the MSE of AR contains 38 geometrid species belonging to 26 genera collected in the above-mentioned regions.

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MATERIALS AND METHODS

The geometer moths of the Azerbaijan part of the Lesser Caucasus were collected mainly by A.Bogachev, R.Effendi, S.Aliyev, and A.Piriyev from Nakhchivan AR (Nakhchivan, Ordubad, Julfa, Shahbuz, Babek, Sharur), Ganja, Kazakh, Tovuz, Aghstafa, Gadabay, Kalbacar, Fizuli, Lachin, Aghdam, Zengilan and Shusha (Fig.). According to the labels, the species were collected in a light trap. Species were identified according to different keys (Lampert, 1913; Romanoff, 1884-1901; Radde, 1899; Viydalep, 1991).

Butterflies are collected by entomological method - during the day through a hand net, and at night from light sources using the wood stain impregnated with ethyl acetate. After death, they are placed on cotton mattresses. In the laboratory, the wings are straightened and they are placed in the collection after identification.

RESULTS AND DISCUSSION

The modern names of the districts on labels:

Yelenendorf, Xanlar – Goygol

Yelizavetpol, Kirovabad – Ganja

Abbreviations: GNM – Georgian National Museum

Family *Geometridae* Leach, 1815

Subfamily *Ennominae* Duponchel, 1845

Genus *Abraxas* Leach, 1815

Abraxas grossulariata Linnaeus, 1758

Material: Azerbaijan - Yelenendorf v. Hajikend, Guetchnan, VI-VIII (Romanoff, 1885), A.Boqacev 1♂; Nakhchivan AR v. Buzqov, 29.05.1974, R.Effendi 1♂, Goygol v. Hajikend, 06.06.2021, S.Hajiyeva 2♂.

Distribution: Wester, Eastern and Middle Europa, Caucasus, Transcaucasia, Crimea, Southem Siberia, China, Japan.

Genus *Tephrina (İsturgia)* Hübner, 1823

Tephrina (İsturgia) arenacearia Denis et Schiffermüller, 1775

Material: Azerbaijan - Nakhchivan AR, Yelizevatopol, Yelenendorf, Darychichek, VI (Romanoff, 1885)**,** Kirovabad, 12.09.1939, A.Boqacev, 1♂; 29.05.1974, Nakhchivan AR v. Buzqov, R.Effendi 1♂; Ganja, 02.06.2021, S.Hajiyeva 1♂ 2♀.

Distribution: Southern Europe, Caucasus, Transcaucasia, Wednesday, Southern Siberia, Primorye, Mongolia.

Genus: *Nychiadea* Lederer, 1853

Nychiades divergaria Staudinger, 1892

Material: Azerbaijan - Nakhchivan AR, v. Kuku, 15.07.1970, R.Effendi 1♂.

Distribution: İran, Azerbaijan, Turkiye.

Nychiades waltheri Hübner, 1813

Material: Azerbaijan - Nakhchivan AR v. Kuku, 15.07.1970, R.Effendi 1♀; Nakhchivan AR, v. Kuku, 18.05.1990 A.Piriyev 2♀ 1♂.

Distribution: Soviet Primorye, Korea, Japan.

Genus *Dycia* Hübner, 1825

Dyscia conspersaria Denis et Schiffermüller, 1775

= *Dyscia conspersaria* (Fabricius, 1775)

Material: Azerbaijan - Yelenendorf v. Garayazı (Romanoff, 1885; Radde, 1899); Karabakh, Nakhchivan AR Ordubad, 1♂ 1♀ (GNM), Ganja v. Hajikend 23.07.2019 S. Hajiyeva 1♂.

Distribution: Southern Europa, Southern Urals, Mid Asia.

Dycia fagaria Hubner, 1813

Material: Azerbaijan - Nakhchivan AR Ordubad v. Bilev 23.05.1957, Zagulyaev 1♀, v. Garmachatakh, 15.06.1967, S.Aliyev, 1♀; Nakhchivan AR, v. Buzgov, 18.05.1974 R.Effendi 1♂; Goygol v. Asigli 05.06.2019 S.Hajiyeva 1♂,

Distribution: Europe, Eastern Ural, Asia Minor.

Dycia sicanaria Obertur, 1923

Material: Azerbaijan - Nakhchivan AR v. Buzgov, R.Effendi 2♂♂, Zengilan v. Vejneli, 15.05.1982, R.Effendi 4♂♂ 2♀♀.

Distribution: South. Europ. h. Russia, Crimea, Caucasus, Transcaucasia, North. Kazakhstan.

Dycia innocentaria christoph, 1885

Material: Azerbaijan - Zengilan v. Vejneli, 19.05.1982, R.Effendi 2♂♂; Ganja v. Hajikend 03.06.2019, S.Hajiyeva 1♂.

Distribution: Caucasus, Transcaucasia, Armenia.

Dycia crassipunctaria Rebel, 1825

Material: Azerbaijan - Nakhchivan AR, v. Buzgov, R.Effendi 2♂♂, Zengilan v. Vejneli, 20.05.1970, R.Effendi 3♂♂.

Distribution: Caucasus, Transcaucasia, Armenia.

Genus *Perconia* Hübner, 1825

Perconia strigillaria Hübner, 1825

Material: Azerbaijan - Ordubad (Alferaki, 1875), GNM 1♂; Nakhchivan AR v. Kuku, 18.05.1970, R.Effendi 2♀ 1♂.

Distribution: European, Southern Siberia, Caucasus, Ural, Mid Asia, Yakutiya, Mongolia, Reserve and Middle Siberia.

Genus *Siona* Dupenchel, 1829

Siona lineata Scopoli, 1763

Material: Azerbaijan - Darychichek (Yelenendorf), Guetchinan (Ordubad), IV-VI (Romanoff, 1885, GNM), Kelbecer v. Yanshakh 29.06.1965, R.Effendi 2♂1♀, Nakhchivan AR v. Bicenek, 12.07.1974, R.Effendi 1♂.

Distribution: Western, Mid. and Eastern Europa, Crimea, Caucasus, Kazakhstan, South Siberia, Amur region, Primorye, Mongolia, Mid Asia.

Genus *Ligdia* Guenne, 1857

Ligdia adustata Denis et Schiffermüller, 1775

Material: Azerbaijan - Elenendorf Darychichek, VI-VII (Romanoff 1885), Ganja v. Hajikend 17.07.2021, S.Hajiyeva 2♂ 1♀.

Distribution: South of the European part of Russia, Caucasus, Crimea, Southern Caucasus, Asia Minor, Iran.

Genus *Synopsia* Hübner, 1825

Synopsia sosiaria Hübner, 1799 (= *S.unitaria* Brout, 1915)

Material: Azerbaijan - Nakhchivan AR v. Buzgov 20.05.1970, R.Effendi 2♂, v. Kuku 09.06.1973, R.Effendi 2♂ .

Distribution: Southern Europa, Caucasus, Mid Asia.

Genus *Chariaspilates* Wehrli, 1825

Chariaspilate formosaria Eversmann, 1953

Material: Azerbaijan - Nakhchivan AR Ordubad 22.06.1973, E.Didmanudze 4♂♂, (GNM); Ganja Hajikend 12.07.2021, S.Hajiyeva 1♂.

Distribution: South European part of Russia, Urals, Northern Kazakhstan, South Sibirian, Amur region, Primopye, Kurile Islands, Mongolia, China, Korean peninsula, Japan.

Genus *Semiothisa* Hübner, 1818

=*Chiasma* Hübner, 1823

=*Diastiotis* Hübner, 1823

=*Jsturgia* Hübner, 1823

=*Macaria* Curtis, 1825

Semiothisa aesthimaia Hübner, 1809

Material: Azerbaijan - Nakhchivan AR - Ordubad IV-VI (Romanoff, 1885), (GNM); Nakhchivan AR v. Julfa 18.06.1973, E.Didmanidze 1♂.

Distribution: South European part of Russia, Crimea, Transcaucasia, Mid Asia, Iran, Afghanistan.

Semiothisa notata Linnaeus, 1758

Material: Azerbaijan - Yelizavetapol Goygol 07.1913, 4♂♂ (GNM); Nax. AR Ordubad IV-VI (Romanoff, 1885), (GNM); Goygol, v. Ashigli, 08.07.2021, S. Hajiyeva 2♂♂.

Distribution: Western, Mid and Eastern Europa, Caucasus, Transcaucasia, Asia Minor, Iran, Afghanistan

Semiothisa clathrata Linnaeus, 1758

Material: Azerbaijan - Yelizavetapol Goygol, 07.1913, 4♂♂ (GNM); Goygol v. Hajikend 12.07.2021, S. Hajiyeva 2♂.

Distribution: Wester, Eastern and Middle Europa, Caucasus, Transcaucasia, Kazakhstan, China, Japan.

Semiothisa lutirata Clerck, 1759

Material: Azerbaijan - Nakhchivan AR Ordubad, 04.07 (Romanoff, 1885), 17.05.1974 1♂; Julfa, 18.06.1973, R.Effendi 1♂.

Distribution: Wester, Eastern and Middle Europa, Western Siberian, Northern Kazakhstan, Caucasus, Transcaucasia.

Semiothisa artesiaria Denis & Schiffermüller, 1775

Material: Azerbaijan - Yelenendorph Varvara l♂ l♀; (Romanof, 1885) GNM; Goygol v. Hajikend 12.07.2021, S.Hajiyeva l♂.

Distribution: Northem, average and Middle Europa, Caucasus, Kazakhstan, south Siberia, Amur region.

Genus *Mannia* Prout, 1915

Mannia oppositaria Mann, 1864

=Tephronia oppositaria Mann, 1864

Material: Azerbaijan - Nakhchivan AR Julfa, 18.06.1973, E.Didmanidze 1♂ 1♀; Goygol v. Ashigli, 05.06.2021, S.Hajiyeva 2♂♂.

Distribution: South Europa, Caucasus, Asia Minor.

Genus *Peribatodes* Wehrli, 1943

Peribatodes rhomboidaria Denis & Schiffermüller, 1775

Material: Azerbaijan - Yelizavetapol Goygol, 07.1913, 4♂♂ (GNM); Nakhchivan AR Julfa, 18.06.1973 E.Didmanidze 1♂ 1♀; Goygol v. Ashigli 05.06.2021, S.Hajiyeva 2♂♂.

Distribution: South Europa, Crimea, Caucasus, Transcaucasia, West. Middle Asia, Altai, Northem Iran.

Peribatodes umbraria Hübner, 1809

Material: Azerbaijan - Nakhchivan AR Ordubad v. Keleki 23.05.1970 R.Effendi 2♂ 1♀; 10.05.1974 Zengilan v. Vejneli R.Effendi 2♂♂; Goygol v. Ashigli 05.06.2021, S.Hajiyeva 2♂♂.

Distribution: Crimea, Caucasus, Transcaucasia, West Middle Asia, Altai, Northem Iran, Afghanistan.

Genus *Biston* Leach, 1815

Biston betularis Linneaus, 1758

= Biston huberaria (Ballion, 1866).

Material: Azerbaijan - Hajikend, 06.1910, 2♂♂ 1♀; Yelizavetapol, Goygol, 07.1913 (GNM); Goygol, 05.06.2017, S.Haciyeva 2♂♂.

Note: The species was found in Yelenendrof by K.Romanoff (1885) and A.Radde (1889).

Distribution: Western, Middle and Eastern Europa, Caucasus, Urals, Kazakhstan, Western Siberia, China, Japan, North America.

Biston stratarius Linneaus, 1758

Material: Azerbaijan – Ordubad Isti dara 06.03.1884 1♂, 1♀ (GNM); Goygol v. Hajikend, 10.04.2019, S.Hajiyeva 2♂♂.

Distribution: Western, Middle and Eastern Europa, Caucasus, Transcaucasia, North-West Africa, Minor Asia.

Genus *Costaconvexa* Agenio, 1949

Costaconueva polygrammata Borkhauseny, 1794

Material: Azerbaijan, Yelenendorph, Varvara, Goytapa, 10.07.1915 (Romanoff, 1887).

Distribution: South Europa, the Crimea, Caucasus, Mid Asia.

Genus *Orthonama* Ectropis

Orthonama obstipata Fabricius, 1794

=Geometra fluviata Hübner, 1799

Material: Azerbaijan – Yelenendorph Gankunda, VI-VII (Romanoff, 1887), GNM.

Distribution: South and Central Europa, Crimea, Sakhalin, Caucasus, Mid Asia.

Genus *Scotopteryx* Hübner, 1825

Scotopteryx bipunctaria (Denis & Schiffermüller, 1775)

=Oetholicha bipunctaria (Denis & Schiffermüller, 1775)

Material: Azerbaijan - Yelizavetapol Goygol, 07.1913, 2♂♂ 2♀♀, (GNM), Goygol v. Hajikend 14.07.2017, S.Hacıyeva 2♂.

Distribution: South and Central Europa, Urals, Caucasus.

Genus *Hylaea* Hübner, 1822

(=*Ellopia* Treitschke, 1825)

Hylia fasciaria Linnaeus, 1758 (= *prosapiaria* Linnaeus, 1758)

Material: Azerbaijan - Azerbaijan, Yelizavetapol Kasikaparan 21.07.1887, Goygol v. Ashigli, 14.07.2017, S.Hacıyeva 2♂♂.

Distribution: Western, Middle and Eastern Europa, Caucasus, Altay, Yakutiya.

Genus *Ascotis* Hübner, 1823

(=*Himera* Düpenchel, 1829)

Ascotis selenaria (Linnaeus, 1761)

Material: Azerbaijan - Goygol v. Ashigli 12.07.2017, S.Hacıyeva 2♂♂ 1♀.

Distribution: Noth, Middle and Eastern Europa, Caucasus, Transcaucasia, Middle Asiya, Noth of Iran.

Genus *Colotois* Hübner, 1825

Colotois pennaria Hübner, 1822

Material: Azerbaijan - Gorge.r. Arax, 17.10.1974, R.Effendi, 2♂♂; Goygol v. Ashigli 9.06.2021, S.Hacıyeva 2♂♂.

Distribution: Spare, Med. and Eastern Europa, Caucasus, Transcaucasia, West Middle

Asiya, Asiya Minor, Noth of America.

Genus *Ennomos* Treitschke, 1825

Ennomos erosaria (Denis & Schiffermüller, 1775)

Material: Azerbaijan, Goygol v. Ashigli, 09.06.2021, S.Hacıyeva, 1♂ 2♀.

Distribution: Western, Middle and Eastern Europa, Caucasus, Transcaucasia, North of Africa.

Genus *Ectropis* Hübner, 1825

Ectropis luridata Borkhausen, 1794 (=extersaria Hübner, 1799)

Material: Azerbaijan - Yelenendorph, Gankunda, VI (Romanof, 1885), Ordubad (Kubistek), 1888 2♂♂, (GNM.), Goygol v. Hacikend 24.06.2019, S.Hacıyeva, 1♂.

Distribution: Western, Middle and Eastern Europa, Caucasus, Transcaucasia.

Genus *Campaea* Lamarck, 1816

=*Metacampa* Latreilla, 1825

Campaea margaritata (Linneaus, 1767)

= *Metrocampa margaritata* Linneaus, 1767

Distribution: Middle and Southern Europa, Caucasus.

Material: Azerbaijan - Yelizavetapol, v. Hajikend, 05.1916, 1♂; Göygöl, 07.1913, 1♀ (GNM); Göygöl, Hajikend 05.07.1919, S.Hacıyeva 1♀.

Genus *Alcis* Curtis, 1825

Alcis jubata Fhunberg, 1788 (= glabraria Hübner, 1790)

Material: Azerbaijan - Yelizavetapol, v. Hajikend, 02.07.1910, 2♂♂ (GNM); Goygol v. Hacikend 05.07.2019, Goygol v. Ashigli, 09.06.2021 S.Hacıyeva, 1♂ 2♀.

Distribution: North, Middle Europa, Ural, Siberia, Amur region, Seaside mountains, Saxalin, Kuril islands, Mongolia, Japan.

Alcis repandata Linnaeus, 1761

Material: Azerbaijan - Yelizavetapol v. Hajikend, 02.09.1910, 2♂♂ (GNM); Goygol v. Hacikend 05.09.2019, S.Hacıyeva, 1♂.

Distribution: North, Middle Europa, Caucasus, Transcaucasia, Altay.

Genus *Gnophos* Treischke, 1827

Gnophos onustaria Hübner, 1853

Material: Azerbaijan - Yelenendorf Istisu, (Romanoff, 1885; Radde, 1899), Fizuli 01.09.1988 A. Priyev 1♂.

Distribution: Southern Caucasus, Western Asia Minor.

Gnophos obscuraria Hübner, 1853.

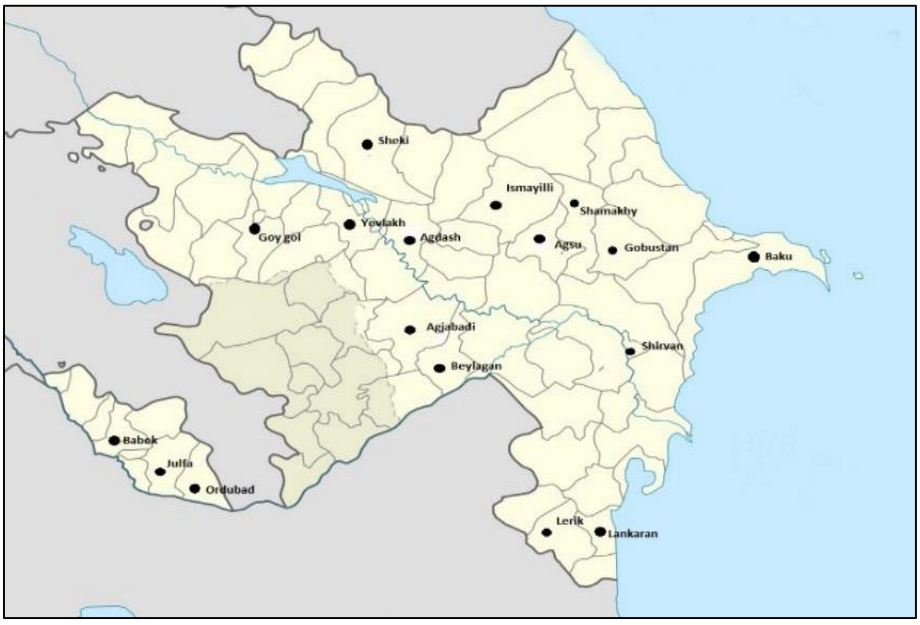

Fig. Records of geometrids in Azerbaijan, localities (filled circles).

Material: Azerbaijan - Lake Gokcha VI-VII (Romanoff, 1885, Yelizavetopol lake Goygol, 07.1913 1♂ (GNM), Goygol 09.06.2021, S.Haciyeva, 2♂.

Distribution: Europa, Asia Minor (North Turkiye).

Gnophos sartata Treitschke, 1827

Material: Azerabaijan - Yelenendorf Istisu (Radde, 1899) (GNM); Naxçıvan AR, v. Buzgov 19.05.1970, R.Effendi, 2♂, Goygol v. Hacikend 24.09.2019, S.Hacıyeva 2♂.

Distribution: South of the European part of Russia, Caucasus, Transcaucasia, Asia Minor.

Gnophos pseudosnelleni Rjabov, 1964

Material: Azerbaijan - Arpachay 06.07.1937 Ryabov (GNM); Naxçıvan AR, v. Buzqov 19.05.1970, R.Effendi 1♀.

Distribution: Transcaucasia, Western. Wed. Asia.

Genus *Personia* Hübner, 1823

Personia strigillaria Hübner, 1789

Material: Azerbaijan - Nakhchivan AR Ordubad, 1875 1♂, (Alferaki, GNM); Goygol, 15.07.1988, A.Piriyev, 2♂♂.

Distribution: Europa, Caucasus, Urals, Middle Siberia, Yakutia, Asia Minor, Mongolia.

Genus *Calospilos* Hübner, 1825

Calospilos sylvato Scopoli, 1767

Material: Azerbaijan - Nakhchivan AR v. Bichenek, 05.06.1964, R.Effendi 1♂, Goygol v. Ashigli, 09.06.2021, S.Hacıyeva, 1♂.

Distribution: Western, Middle and Eastern Europa, Caucasus, Crimea, Siberia reserve, China, Japan.

Among 26 genera of *Ennominae* subfamily belonging to geometer moths of the Lesser Caucasus and Middle Araz region of Azerbaijan, *Abraxas, Thephrina, Perconia, Siona, Ligdia, Synopsia, Chariaspilares, Mannia, Costaconvexa, Ortonama, Scotopteryx, Hylaea, Ascotis, Campaea, Personia, and Calos*pilos are represented by one species, the genera *Nychiodes, Peribatotes*, *Biston*, and *Alcis* by 2 species, the genus *Gnopho*s by 4 species, the genera *Dysia* and *Semiothisa* by 5 species. The genera *Semiothisa* and *Gnophos* predominate in terms of the number of species.

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Study of single nucleotide polymorphism in the encoding exons of the AGT gene of an arterial hypertension patient from Azerbaijan

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Arterial hypertension, which affects 20-30% of the world's population, is one of the main causes of cardiovascular disease. According to existing ideas, arterial hypertension is caused by genetic and non-genetic reasons, however, in most cases (more than 90%), the specific cause (s) of this complication in essential hypertension is unknown. It is believed that blood pressure is under the control of numerous genes. One such gene is the AGT gene, which encodes the angiotensinogen/angiotensin protein hormone. To date, a total of 4129 SNPs for the AGT gene have been annotated in the dbSNP database https://www.ncbi.nlm.nih.gov/snp/?term=AGT). In this study, the nucleotide sequences of the 3rd, 4th, 5th and 6th coding exons of the AGT gene in a sample of Azerbaijani patients living in Azerbaijan diagnosed with high arterial hypertension were read and compared with the corresponding reference sequences. It was found that there are 8 mutations in these exons, and all of them have been annotated in dbSNP resources. The results of studying the possible functional role of these mutations are interpreted below.

Keywords: *Human, arterial hypertension (AH), angiotensin (AGT) gene, exon analysis, single nucleotide polymorphism (SNP)*

INTRODUCTION

Arterial high (140/90 mm Hg and above) blood pressure (hypertension), which affects 20-30 percent of the world's population, is one of the main causes of cardiovascular disease. Studies conducted to date indicate that the cause of arterial hypertension (AH) is genetic and non-genetic factors (environment, nutrition and stress). There are two types of hypertension: (1) essential hypertension (primary hypertension, idiopathic hypertension), the specific causes of which are unknown and affect more than 90 percent of people whose specific causes are unknown and who suffer from this pathology, and (2) secondary hypertension caused by known diseases and causes such as pathologies kidneys or lungs, stress, diet, excess weight, smoking, etc. (O'Rourke, 2003; Nguen and Jaisser, 2012; Kunutsor and Powles, 2010; Gruesser et al., 2007; Artman et al., 2007; for summary: Messerley, Williams and Ritz, 2007; Jordan, Kurschat and Reuter, 2018). Existing knowledge confirms that it would be wrong to consider all cases of arterial hypertension as essential (primary) hypertension.

Arterial pressure is estimated to be controlled by numerous genes, and in most known cases, hypertension manifests itself as a feature of non-Mendelian syndrome (Delles et al., 2010; Austin and Loyd, 2014; Garcia-Rivas et al., 2017; Morrell et al., 2019). To date, more than 40 genes have been detected related to hypertension (see https://www.omim.org/entry/145500).

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For example, AGT, which encodes the renin-angiotensin-aldosterone-related hormone hypertensogen / angiotensin protein Jeunemaitre et al., 1992; Caulfield et al., 1994; Lifton, 1996), AGTR1 (Bonnardeaux et al., 1994), which encodes the first type of receptor of the angiotensin II protein, ACE, which encodes an angiotensin-converting enzyme (Zhang et al., 2004), the APA gene, which encodes aminopeptidase (Ferreira and Raizada, 2008), REN, which encodes the enzyme renin (Persson, 2003; Wu et al., 2018), and MTHFR encoding the enzyme methylenetetrahydrofolate reductase (McNulty et al., 2016; https://www.omim.org/entry/607093).

Angiotensin I secreted by the liver undergoes successive transformations by renin and angiotensin-converting enzyme, resulting in the active hormone angiotensin II, which raises blood pressure (Lifton, 1996). There are significant differences in the concentration of angiotensinogen in the blood plasma of arterial hypertension patients with different AGT genotypes (Jeunemaitre et al., 1992; Caulfield et al., 1994). The AGT gene is mainly expressed in the liver hepatocytes. An angiotensinogen from α globulin family is a 485 amino acid (at) long protein that is the precursor of the angiotensin II protein and the sole substrate of the renin protein. After cleavage of the signal peptide, 452 a.a. mature angiotensinogen protein is formed. The mature form of the angiotensinogen protein is the sole substrate of the renin protein, which consists of 340 a.a.: after the peptide bond between Leu and Val amino acids is cut by renin, the 452 a.a. protein is converted to the 10 a.a. peptide hormone - angiotensin I (Asp-Arg-Val-Tyr - Ile-His-Pro-Phe-His-Leu). The ACE enzyme then converts the angiotensin I peptide to the 8 amino acid (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) angiotensin II peptide hormone. Besides, the enzyme aminopeptidase A cleaves the angiotensin II peptide to 7 a.a. peptide hormone angiotensin III (Arg-Val-Tyr-Ile-His-Pro-Phe), and then the peptide angiotensin III to 6 a.a. peptide hormone angiotensin IV (Val-Tyr-Ile-His-Pro-Phe). The separate biological function of angiotensin I peptide is unknown. Angiotensin III peptide has approximately 40% of the antihypertensive activity of angiotensin II peptide. Angiotensin IV hormone is characterized by the least antihypertensive properties but has a wide range of activities in the central nervous system. Circulating levels of angiotensinogen in the body are regulated by various stimuli, including glucocorticoids, estrogens, thyroid hormone, insulin, and some cytokines (Lifton, 1996; Ferreira and Raizada, 2008; Lu et al., 2016; Wu et al., 2018; Chappell, 2019).

AGT gene with a total length of 43061 bp, including introns, is located on the opposite (negative) strand of DNA [complement (230702523..230745583)] on human chromosome 1, consists of 6 exons. Since the transcription of this gene is initiated from alternative TSSs, this results in partially 2 different mRNAs but single DNA coding sequence (CDS) (NC_000001.11; https://www.genecards.org/cgi-bin/carddisp.pl?gene=AGT&keywords=AGT; Fig. 1). As a result of the translation of this CDS, the initial (precursor) form of angiotensin polypeptide - angiotensinogen - is formed.

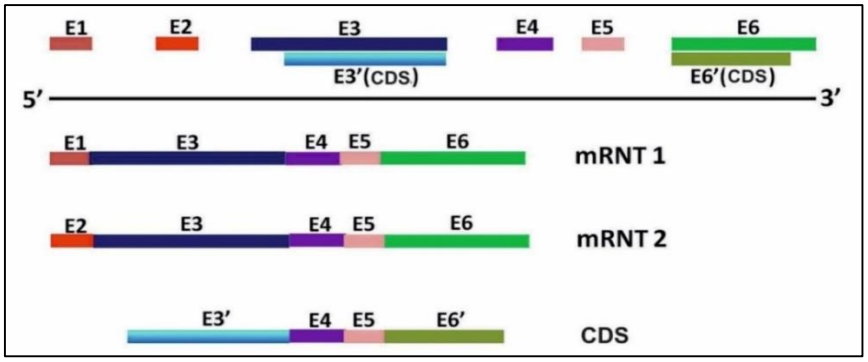

Fig. 1. Exon-intron structure of AGT gene. Localization of exons: E1: c(230745515.. 230745583), E2: c(230714086..230714122), E3: c(230709995..230710853), E4: c(230705933.. 230706200), E5: c(2307.04193). 230704337), E6: c(230702523..230703329). The parts of E3 and E6 exons included in CDS were marked as "E3'" and "E6'"respectively. E3': c(230709995.. 230710823), E6': c(230703141.. 230703329).

In the DNA sequence of the human AGT gene (CDS), the T>C mutation (in the Met268Thr or M268T protein; SNP M235T or rs699 in the scientific literature in dbSNP resources) at position 803 (codon 268) is associated with cardiovascular disease (Mohammadi et al., 2018). For example, in people with arterial hypertension, the frequency of 235T/T genotype and 235T allele is relatively high compared to the control group, and these people are included in the risk group (Cheng, Wang and Wan, 2012; Borai et al., 2018; Azova et al., 2021). On the other hand, it has been noted that people with 235M/M genotype and 235M allele of the AGT gene are in the risk group for high blood pressure (Kim et al., 2015). The 28A>T mutation in exon 2 may play a role in damage to the AGT protein. 90T>C mutation in the 3'-untranslated region (TOR) severely affects mRNA stability and translation (Padma et al., 2015).

In total, 4129 SNPs (3862 substitutions and 267 insertions; https://www.ncbi.nlm.nih.gov/snp/?term=AGT) have been annotated in the dbSNP database so far in the AGT gene.

In this study, the nucleotide sequences of the 3rd, 4th, 5th and 6th coding exons (E3, E4, E5 and E6) of the AGT gene were read in a sample of Azerbaijani patients living in Azerbaijan and diagnosed with high AH, and their SNP spectrum was analyzed by comparison with relevant reference sequences. The results of these studies are interpreted below.

MATERIALS AND METHODS

For the study, one of 127 patients (27 women and 65 men under 35 years old, 20 women and 15 men over 35 years old) were randomly selected for treatment with a diagnosis of hypertension at the Research Institute of Cardiology named after J.M.Abdullayev. The total DNA fraction from the blood sample of the selected patient was isolated using the "QIAamp DNA Mini Kit, Blood Mini (50)" reagent set (catalog no. 51104) manufactured by the QIAGEN company (Germany). The obtained blood sample was first lysed by incubating with proteinase K at 56 ºC for ~2 hours in a buffer containing SDS and EDTA. The lysis process was completed by adding AL buffer and incubating at 70 ºC for ~10 min. The reaction mixture was then vortexed by the addition of 200-400 µl (96%) ethanol. The resulting mixture was transferred to a mini-spin column and centrifuged at 6000 g for 1 minute, the remaining mixture after discarding the supernatant was transferred to a new tube, and washed with 500 µl of washing buffer (Buffer AW2) by centrifugation at 20000 g for 3 minutes. Then, the column was transferred to a new tube to remove the remnants of the washing buffer and centrifuged at 20000 g for 1 minute. To elute the obtained total DNA fraction from the spin column, it was transferred to a new (1.5 ml) tube and incubated for 1-2 min at room temperature with the addition of 200 µl AE buffer (10 mM Tris·Cl; 0.5 mM EDTA; pH 9.0). for DNA solubilization) and centrifuged at 6000 g for 1 min. Finally, the concentration of the resulting total DNA fraction was determined and stored at –20 ºC until use.

The following oligonucleotide primers were used to sequence 4 exons of the AGT gene (E3 and E3', E4, E5, E6) in the total DNA fraction. E3:5′-GGTATGCGGAAGCGAGCAC-3′; E3':5′-CTTGGAAGTGGACGTAGGT-3′; E4:5′-GGAAGATGAAGGGCTTCTC-3′; E5:5′-GACCATCCACCTGACCATG-3′; E6:5′-TGCTTTGCAGGTGCTGAAC-3′.

Exon nucleotide sequences were read using the Sanger method at the Afgen Genetic Diagnostic Center operating at the Baku Clinic of Biological Medicine using a Genetic Analyzer 3130xl manufactured by Applied Biosystems.

The localization of exons of the AGT gene and reference nucleotide sequences, as well as data on the reference amino acid (at) sequence of the corresponding protein, were taken from the GRCh38.p13 (NC_000001.11) version of the GenBank annotation (Refseq) of the human genome, and information on the localization and functional role of known SNPs in these exons was taken from the dbSNP resources (https://www.ncbi.nlm.nih.gov/snp/?term=AGT).

The comparison of nucleotide and amino acid sequences was carried out using the **BLAST** (Altschul S.F. et al. (1997) software package, the study of the distribution spectrum in terms of the number and functional significance of known SNPs in exons was carried out using the computer

program **getsnpp2** (I.Shahmuradov, unpublished). All bioinformatic analyses were implemented in the LINUX operating system.

RESULTS AND DISCUSSION

Nucleotide sequences of the newly sequenced coding exons of the AGT gene and their comparison with the corresponding reference sequences

The nucleotide sequence of 4 exons (E3-E6) included in the CDS of the AGT gene was sequenced and compared with the relevant reference sequences (figures 2-5). There are 2 point mutations (SNP - substitution) between each of these exons and the relevant reference exon: C>A (G>T in the complementary coding strand, dbSNP ID: rs61762539) at position 415 (chromosome 230710439) on exon 3 and G>A (C>T; rs545870660) transversions at position 607 (230710284), G>A (C>T; rs61762530) transition at position 33 (230706168) of exon 4, and at position 260 T>A (A>T; rs7080) transversion (230705941), C>T (G>A) transitions at position 98 (230704240) and at position 137 (230704201) of exon 5; rs886046081 and rs61751067), A>G (T>C; rs5043) transition at position 366 (230702974; rs5043) and G>T (C>A; rs7079) transversion at position 755 (230702585) of exon 6. These SNPs are found within the CDS in exons 2, 3, and 4 and in the 3'-UTR region in exon 6

```
AGT_E3    1  GGTATGCGGAAGCGAGCACCCCAGTCTGAGATGGCTCCTGCCGGTGTGAGCCTGAGGGCC
AGT_E3r   1  ............................................................

AGT_E3   61  ACCATCCTCTGCCTCCTGGCCTGGGCTGGCCTGGCTGCAGGTGACCGGGTGTACATACAC
AGT_E3r  61  ............................................................

AGT_E3  121  CCCTTCCACCTCGTCATCCACAATGAGAGTACCTGTGAGCAGCTGGCAAAGGCCAATGCC
AGT_E3r 121  ............................................................

AGT_E3  181  GGGAAGCCCAAAGACCCCACCTTCATACCTGCTCCAATTCAGGCCAAGACATCCCCTGTG
AGT_E3r 181  ............................................................

AGT_E3  241  GATGAAAAGGCCCTACAGGACCAGCTGGTGCTAGTCGCTGCAAAACTTGACACCGAAGAC
AGT_E3r 241  ............................................................

AGT_E3  301  AAGTTGAGGGCCGCAATGGTCGGGATGCTGGCCAACTTCTTGGGCTTCCGTATATATGGC
AGT_E3r 301  ............................................................

AGT_E3  361  ATGCACAGTGAGCTATGGGGCGTGGTCCATGGGGCCACCGTCCTCTCCCCAACGtCTGTC
AGT_E3r 361  ............................................................g.....

AGT_E3  421  TTTGGCACCCTGGCCTCTCTCTATCTGGGAGCCTTGGACCACACAGCTGACAGGCTACAG
AGT_E3r 421  ............................................................

AGT_E3  481  GCAATCCTGGGTGTTCCTTGGAAGGACAAGAACTGCACCTCCCGGCTGGATGCGCACAAG
AGT_E3r 481  ............................................................

AGT_E3  541  GTCCTGTCTGCCCTGCAGGCTGTACAGGGtCTGCTAGTGGCCCAGGGCAGGGCTGATAGC
AGT_E3r 541  ............................c...............................

AGT_E3  601  CAGGCCCAGCTGCTGCTGTCCACGGTGGTGGGCGTGTTCACAGCCCCAGGCCTGCACCTG
AGT_E3r 601  ............................................................

AGT_E3  661  AAGCAGCCGTTTGTGCAGGGCCTGGCTCTCTATACCCCTGTGGTCCTCCCACGCTCTCTG
AGT_E3r 661  ............................................................
```

Figure 2 continued

```
AGT_E3   721  GACTTCACAGAACTGGATGTTGCTGCTGAGAAGATTGACAGGTTCATGCAGGCTGTGACA
AGT_E3r  721  ............................................................

AGT_E3   781  GGATGGAAGACTGGCTGCTCCCTGATGGGAGCCAGTGTGGACAGCACCCTGGCTTTCAAC
AGT_E3r  781  ............................................................

AGT_E3   841  ACCTACGTCCACTTCCAAG
AGT_E3r  841  ...................
```

Fig. 2. Comparison of the nucleotide sequence of the 3rd exon (E3) of the AGT gene in a hypertensive patient belonging to the Azerbaijani population with the reference sequence of this exon. Here and in the following figures, rreference sequences, dots denote the same nucleotides. Here, as in Figures 3, 4 and 5, SNPs are marked with small and red letters on a gray background. The translation initiation codon is marked in pink.

```
AGT_E4     1  GGAAGATGAAGGGCTTCTCCCTGCTGGCCGAGtCCCAGGAGTTCTGGGTGGACAACAGCA
AGT_E4r    1  ..................................c............................

AGT_E4    61  CCTCAGTGTCTGTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCC
AGT_E4r   61  ............................................................

AGT_E4   121  AGGACAACTTCTCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCC
AGT_E4r  121  ............................................................

AGT_E4   181  AGCCTCACTATGCCTCTGACCTGGACAAGGTGGAGGGTCTCACTTTCCAGCAAAACTCCC
AGT_E4r  181  ............................................................

AGT_E4   241  TCAACTGGATGAAGAAACTtTCTCCCCG
AGT_E4r  241  ..................a.........
```

Fig. 3. Nucleotide sequence of the 4th exon of the AGT gene of a hypertensive patient belonging to Azerbaijani population and comparison with the reference sequence of that exon.

```
AGT_E5     1  GACCATCCACCTGACCATGCCCCAACTGGTGCTGCAAGGATCTTATGACCTGCAGGACCT
AGT_E5r    1  ............................................................

AGT_E5    61  GCTCGCCCAGGCTGAGCTGCCCGCCATTCTGCACACCaAGCTGAACCTGCAAAAATTGAG
AGT_E5r   61  ........................................g...................

AGT_E5   121  CAATGACCGCATCAGGaTGGGGGAG
AGT_E5r  121  ................g........
```

Fig. 4. Nucleotide sequence of the 5th exon of the AGT gene of a hypertensive patient belonging to the Azerbaijani population and comparison with the reference sequence of that exon.

```
AGT_E6     1  TGCTTTGCAGGTGCTGAACAGCATTTTTTTTGAGCTTGAAGCGGATGAGAGAGAGCCCAC
AGT_E6r    1  ............................................................

AGT_E6    61  AGAGTCTACCCAACAGCTTAACAAGCCTGAGGTCTTGGAGGTGACCCTGAACCGCCCATT
AGT_E6r   61  ............................................................

AGT_E6   121  CCTGTTTGCTGTGTATGATCAAAGCGCCACTGCCCTGCACTTCCTGGGCCGCGTGGCCAA
AGT_E6r  121  ............................................................
```

Figure 5 continued

```
AGT_E6    181  CCCGCTGAGCACAGCATGAGGCCAGGGCCCCAGAACACAGTGCCTGGCAAGGCCTCTGCC
AGT_E6r   181  ............................................................

AGT_E6    241  CCTGGCCTTTGAGGCAAAGGCCAGCAGCAGATAACAACCCCGGACAAATCAGCGATGTGT
AGT_E6r   241  ............................................................

AGT_E6    301  CACCCCCAGTCTCCCACCTTTTCTTCTAATGAGTCGACTTTGAGCTGGAAAGCAGCCGTT
AGT_E6r   301  ............................................................

AGT_E6    361  TCTCCcTGGTCTAAGTGTGCTGCATGGAGTGAGCAGTAGAAGCCTGCAGCGGCACAAATG
AGT_E6r   361  .....t......................................................

AGT_E6    421  CACCTCCCAGTTTGCTGGGTTTATTTTAGAGAATGGGGGTGGGGAGGCAAGAACCAGTGT
AGT_E6r   421  ............................................................

AGT_E6    481  TTAGCGCGGGACTACTGTTCCAAAAAGAATTCCAACCGACCAGCTTGTTTGTGAAACAAA
AGT_E6r   481  ............................................................

AGT_E6    541  AAAGTGTTCCCTTTTCAAGTTGAGAACAAAAATTGGGTTTTAAAATTAAAGTATACATTT
AGT_E6r   541  ............................................................

AGT_E6    601  TTGCATTGCCTTCGGTTTGTATTTAGTGTCTTGAATGTAAGAACATGACCTCCGTGTAGT
AGT_E6r   601  ............................................................

AGT_E6    661  GTCTGTAATACCTTAGTTTTTTCCACAGATGCTTGTGATTTTTGAACAATACGTGAAAGA
AGT_E6r   661  ............................................................

AGT_E6    721  TGCAAGCACCTGAATTTCTGTTTGAATGCGGAACaATAGCTGGTTATTTCTCCCTTGTGT
AGT_E6r   721  ..................................c.........................

AGT_E6    781  TAGTAATAAACGTCTTGCCACAATAAGCCTCCAAAAA
AGT_E6r   781  ...................................
```

Fig. 5. Nucleotide sequence of the 6th exon of the AGT gene of a hypertensive patient belonging to Azerbaijani population and comparison with the reference sequence of that exon. The translation termination codon is marked in pink.

Known SNPs in Recently Sequenced Coding Exons of the AGT Gene and Their Possible Functional Significance

Integrative data on the distribution of SNPs collected for AGT gene on the 4 mRNA/CDS exons and their influence on the phenotype in dbSNP resources are indicated in Table 1. In total, 851 and 586 TNPs are known in these 4 exons only for mRNA and KDA, respectively. However, so far only 27 (26) SNPs have been found to have functional consequences: only 4 SNPs have a pathogenic effect, 1 SNP belongs to the risk group, and 22 SNPs had no deleterious effects. In total, 1290 of the 1343 known SNPs in the AGT gene have no known functional consequences.

To assess the possible functional role of SNPs found in the AGT gene in a patient with arterial hypertension belonging to the Azerbaijani population, the nucleotide sequences of newly read and reference variants of the 3rd, 4th, 5th and 6th exons of CDSs and the amino acid sequences of the corresponding proteins were compared (Figure 6).

rs61762539 (C>A, G>T in the complementary DNA strand) variation in exon 3 of the AGT gene results in the replacement of the amino acid arginine (R) with the amino acid serine (S) in the protein sequence. According to dbSNP data, this mutation has some clinical role,

but the specific mechanism of that effect is unknown. Another SNP in exon 3 (rs545870660; G>A, C>T) is a synonymous mutation and does not play any clinical role.

The rs61762530 (G>A, C>T) mutation in exon 4 of the AGT gene results in the substitution of the amino acid proline (P) for the amino acid serine and has a certain clinical role with an unknown specific mechanism of action. Another SNP in this exon (rs7080; T>A, A>T) is synonymous in nature and does not play any clinical role.

Mutation rs886046081 (C>T, G>A) in exon 5 of the AGT gene leads to the replacement of glutamic acid (E) by lysine (K), and mutation rs61751067 (C>T, G>A) leads to the replacement of the amino acid valine (V) to methionine (M). These mutations have a specific clinical role, the mechanism of action of which is unknown.

Table 1. Distribution and functional role of known SNPs on the coding exons E3, E4, E5 and E6 of the AGT gene

Exons (E)	**SNPs**				
	Pathogenic	**Risky**	**Harmless**	**Unknown**	**Total**
mRNT-E3, CDS-E3’	1 (1)	1 (1)	11 (11)	351 (339)	364 (352)
mRNT-E4, CDS-E4	2 (2)	0 (0)	6 (6)	95 (95)	103 (103)
mRNT-E5, CDS-E5	0 (0)	0 (0)	1 (1)	59 (59)	60 (60)
mRNT-E6, CDS-E6’	1 (0)	0 (0)	4 (3)	219 (65)	224 (69)
Total across 4 exons	**4 (3)**	**1 (1)**	**22 (21)**	**724 (558)**	**751 (584)**

```
                  ------> E3
AGT_Protⁿ           M  R  P  R  G  V  S  L  R  R  T  I  L  C  L  L  R  W  R  G
AGT_CDSⁿ       1   ATGGCTCCTGCCGGTGTGAGCCTGAGGGCCACCATCCTCTGCCTCCTGGCCTGGGCTGGC
AGT_CDSʳ       1   ATGGCTCCTGCCGGTGTGaGCCTGAGGGCCACCATCCTCTGCCTCCTGGCCTGGGCTGGC
AGT_Protʳ           M  R  P  R  G  V  S  L  R  R  T  I  L  C  L  L  R  W  R  G

AGT_Protⁿ           L  R  R  G  D  R  V  Y  I  H  P  F  H  L  V  I  H  N  E  S
AGT_CDSⁿ      61   CTGGCTGCAGGTGaCCGGGTGTACATACACCCCTTCCACCTCGTCATCCACAATGAGAGT
AGT_CDSʳ      61   CTGGCTGCaGGTGACCGGGTGTACATACACCCCTTCCACCTCGTCATCCACAATGAGAGT
AGT_Protʳ           L  R  R  G  D  R  V  Y  I  H  P  F  H  L  V  I  H  N  E  S

AGT_Protⁿ           T  C  E  Q  L  R  K  R  N  R  G  K  P  K  D  P  T  F  I  P
AGT_CDSⁿ     121   ACCTGTGAGCAGCTGGCAAAGGCCAATGCCGGGAAGCCCAAAGACCCCACCTTCATACCT
AGT_CDSʳ     121   ACCTGTGAGCAGCTGGCAAAGGCCAATGCCGGGAAGCCCAAAGACCCCACCTTCATACCT
AGT_Protʳ           T  C  E  Q  L  R  K  R  N  R  G  K  P  K  D  P  T  F  I  P

AGT_Protⁿ           R  P  I  Q  R  K  T  S  P  V  D  E  K  R  L  Q  D  Q  L  V
AGT_CDSⁿ     181   GCTCCAATTCAGGCCAAGACATCCCCTGTGGATGAAAAGGCCCTACAGGACCAGCTGGTG
AGT_CDSʳ     181   GCTCCAATTCAGGCCAAGACATCCCCTGTGGATGAAAAGGCCCTACAGGACCAGCTGGTG
AGT_Protʳ           R  P  I  Q  R  K  T  S  P  V  D  E  K  R  L  Q  D  Q  L  V

AGT_Protⁿ           L  V  R  R  K  L  D  T  E  D  K  L  R  R  R  M  V  G  M  L
AGT_CDSⁿ     241   CTAGTCGCTGCAAAACTTGACACCGAAGACAAGTTGAGGGCCGCAATGGTCGGGATGCTG
AGT_CDSʳ     241   CTAGTCGCTGCAAAACTTGACACCGAAGACAAGTTGAGGGCCGCAATGGTCGGGATGCTG
AGT_Protʳ           L  V  R  R  K  L  D  T  E  D  K  L  R  R  R  M  V  G  M  L

AGT_Protⁿ           R  N  F  L  G  F  R  I  Y  G  M  H  S  E  L  W  G  V  V  H
AGT_CDSⁿ     301   GCCAACTTCTTGGGCTTCCGTATATATGGCATGCACAGTGAGCTATGGGGCGTGGTCCAT
AGT_CDSʳ     301   GCCAACTTCTTGGGCTTCCGTATATATGGCATGCACAGTGAGCTATGGGGCGTGGTCCAT
AGT_Protʳ           R  N  F  L  G  F  R  I  Y  G  M  H  S  E  L  W  G  V  V  H

AGT_Protⁿ           G  R  T  V  L  S  P  T  S  V  F  G  T  L  R  S  L  Y  L  G
```

Figure 6 continued

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AGT_CDSⁿ    361  GGGGCCACCGTCCTCTCCCCAACGtCTGTCTTTGGCACCCTGGCCTCTCTCTATCTGGGA
AGT_CDSʳ    361  GGGGCCACCGTCCTCTCCCCAACGgCTGTCTTTGGCACCCTGGCCTCTCTCTATCTGGGA
AGT_Protʳ         G  R  T  V  L  S  P  T  R  V  F  G  T  L  R  S  L  Y  L  G

AGT_Protⁿ         R  L  D  H  T  R  D  R  L  Q  R  I  L  G  V  P  W  K  D  K
AGT_CDSⁿ    421  GCCTTGGACCACACAGCTGACAGGCTACAGGCAATCCTGGGTGTTCCTTGGAAGGACAAG
AGT_CDSʳ    421  GCCTTGGACCACACAGCTGACAGGCTACAGGCAATCCTGGGTGTTCCTTGGAAGGACAAG
AGT_Protʳ         R  L  D  H  T  R  D  R  L  Q  R  I  L  G  V  P  W  K  D  K

AGT_Protⁿ         N  C  T  S  R  L  D  R  H  K  V  L  S  R  L  Q  R  V  Q  G
AGT_CDSⁿ    481  AACTGCACCTCCCGGCTGGATGCGCACAAGGTCCTGTCTGCCCTGCAGGCTGTACAGGGt
AGT_CDSʳ    481  AACTGCACCTCCCGGCTGGATGCGCACAAGGTCCTGTCTGCCCTGCAGGCTGTACAGGGc
AGT_Protʳ         N  C  T  S  R  L  D  R  H  K  V  L  S  R  L  Q  R  V  Q  G
AGT_Protⁿ         L  L  V  R  Q  G  R  R  D  S  Q  R  Q  L  L  L  S  T  V  V
AGT_CDSⁿ    541  CTGCTAGTGGCCCAGGGCAGGGCTGATAGCCAGGCCCAGCTGCTGCTGTCCACGGTGGTG
AGT_CDSʳ    541  CTGCTAGTGGCCCAGGGCAGGGCTGATAGCCaGGCCCAGCTGCTGCTGTCCaCGGTGGTG
AGT_Protʳ         L  L  V  R  Q  G  R  R  D  S  Q  R  Q  L  L  L  S  T  V  V

AGT_Protⁿ         G  V  F  T  R  P  G  L  H  L  K  Q  P  F  V  Q  G  L  R  L
AGT_CDSⁿ    601  GGCGTGTTCACAGCCCCAGGCCTGCACCTGAAGCAGCCGTTTGTGCAGGGCCTGGCTCTC
AGT_CDSʳ    601  GGCGTGTTCACAGCCCCAGGCCTGCACCTGAAGCAGCCGTTTGTGCAGGGCCTGGCTCTC
AGT_Protʳ         G  V  F  T  R  P  G  L  H  L  K  Q  P  F  V  Q  G  L  R  L

AGT_Protⁿ         Y  T  P  V  V  L  P  R  S  L  D  F  T  E  L  D  V  R  R  E
AGT_CDSⁿ    661  TATACCCCTGTGGTCCTCCCACGCTCTCTGGACTTCACAGAACTGGATGTTGCTGCTGAG
AGT_CDSʳ    661  TATACCCCTGTGGTCCTCCCaCGCTCTCTGGACTTCACAGAACTGGATGTTGCTGCTGAG
AGT_Protʳ         Y  T  P  V  V  L  P  R  S  L  D  F  T  E  L  D  V  R  R  E

AGT_Protⁿ         K  I  D  R  F  M  Q  R  V  T  G  W  K  T  G  C  S  L  M  G
AGT_CDSⁿ    721  AAGATTGACAGGTTCATGCAGGCTGTGACAGGATGGAAGACTGGCTGCTCCCTGATGGGA
AGT_CDSʳ    721  AAGATTGACAGGTTCATGCAGGCTGTGACAGGATGGAAGACTGGCTGCTCCCTGATGGGA
AGT_Protʳ         K  I  D  R  F  M  Q  R  V  T  G  W  K  T  G  C  S  L  M  G
                                                   E3 <------ ------> E4
AGT_Protⁿ         R  S  V  D  S  T  L  R  F  N  T  Y  V  H  F  Q  G  K  M  K
AGT_CDSⁿ    781  GCCAGTGTGGACAGCACCCTGGCTTTCAACACCTACGTCCACTTCCAAGGGAAGATGAAG
AGT_CDSʳ    781  GCCAGTGTGGACAGCACCCTGGCTTTCAACACCTACGTCCACTTCCAAGGGAAGATGAAG
AGT_Protʳ         R  S  V  D  S  T  L  R  F  N  T  Y  V  H  F  Q  G  K  M  K

AGT_Protⁿ         G  F  S  L  L  R  E  S  Q  E  F  W  V  D  N  S  T  S  V  S
AGT_CDSⁿ    841  GGCTTCTCCCTGCTGGCCGAGtCCCAGGAGTTCTGGGTGGACAACAGCACCTCAGTGTCT
AGT_CDSʳ    841  GGCTTCTCCCTGCTGGCCGAGcCCCAGGAGTTCTGGGTGGACAACAGCACCTCAGTGTCT
AGT_Protʳ         G  F  S  L  L  R  E  P  Q  E  F  W  V  D  N  S  T  S  V  S

AGT_Protⁿ         V  P  M  L  S  G  M  G  T  F  Q  H  W  S  D  I  Q  D  N  F
AGT_CDSⁿ    901  GTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCCAGGACAACTTC
AGT_CDSʳ    901  GTTCCCATGCTCTCTGGCATGGGCACCTTCCAGCACTGGAGTGACATCCAGGACAACTTC
AGT_Protʳ         V  P  M  L  S  G  M  G  T  F  Q  H  W  S  D  I  Q  D  N  F

AGT_Protⁿ         S  V  T  Q  V  P  F  T  E  S  R  C  L  L  L  I  Q  P  H  Y
AGT_CDSⁿ    961  TCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCCAGCCTCACTAT
AGT_CDSʳ    961  TCGGTGACTCAAGTGCCCTTCACTGAGAGCGCCTGCCTGCTGCTGATCCAGCCTCACTAT
AGT_Protʳ         S  V  T  Q  V  P  F  T  E  S  R  C  L  L  L  I  Q  P  H  Y

AGT_Protⁿ         R  S  D  L  D  K  V  E  G  L  T  F  Q  Q  N  S  L  N  W  M
```

Figure 6 continued

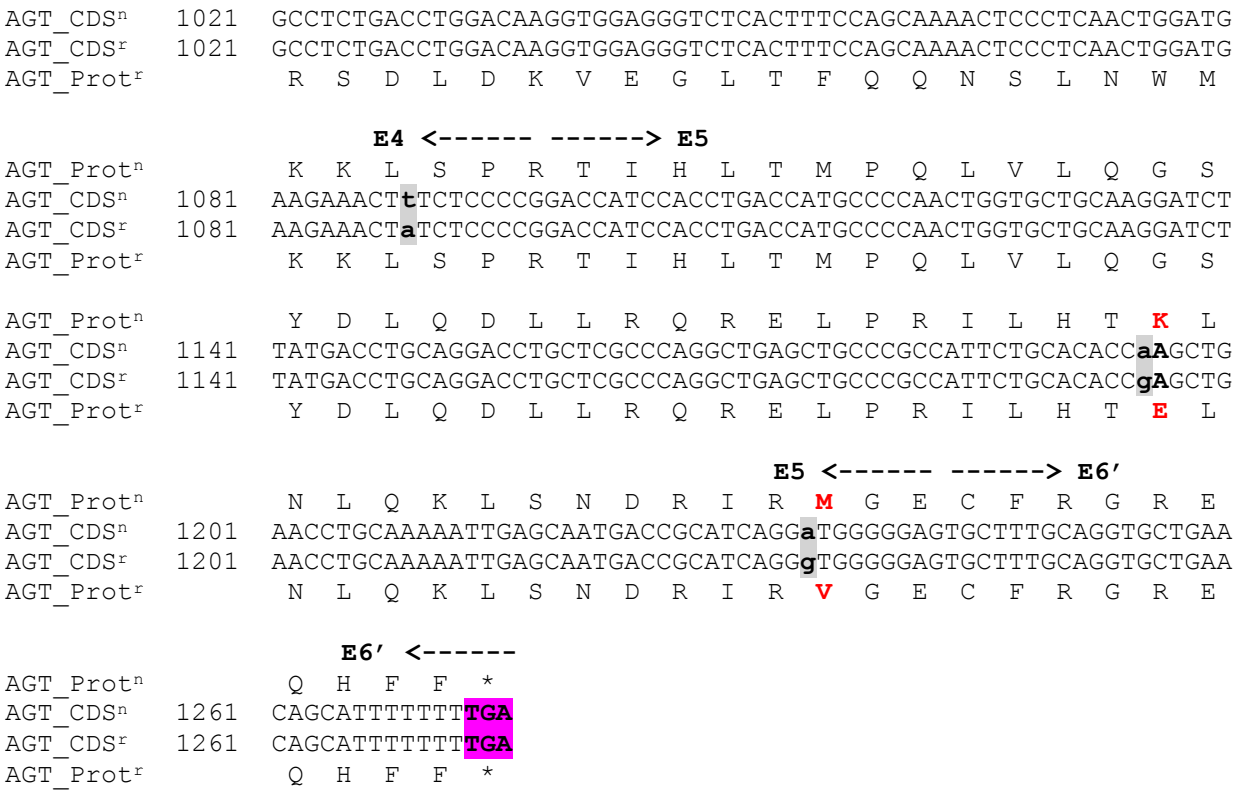

Fig. 6. Comparison of the CDS consisting of the 3rd, 4th, 5th and 6th exons of the AGT gene in a hypertensive patient from Azerbaijan and the nucleotide and amino acid sequences of the corresponding protein with the reference gene and protein sequences. Portions of exons represented in CDS: E3' - 1..830, E4 - 831..1097, E5 - 1098..1242, E6' - 1243..1275. SNPs are shown in gray background, amino acids changed as a result of mutation are shown in red. Translation initiation and termination codons are marked in bold on a pink background.

Despite the fact that mutations rs5043 (A>G, T>C) and rs7079 (G>T, C>A) in the 6th exon of the AGT gene fall into the 3'-UTR region, they have a certain clinical role, which mechanism of action is unknown.

Thus, 6 of the 8 mutations detected in the AGT gene of the hypertensive patient belonging to the Azerbaijani population occurred in the CDS, and 2 occurred in the 3'-UTR region. 4 SNPs causing amino acid substitutions in the protein sequence and 2 SNPs in 3'- UTR have a definite clinical role (according to dbSNP data), while 2 SNPs in CDS are of synonymous character.

It should be noted here that the rs699 SNP (T>C; Met268Thr or M268T; Kim et al., 2015, Mohammed et al., 2018) found in position 803 (codon 268) of the CDS of the AGT gene and associated with cardiovascular disease was not detected in the AGT gene of the hypertensive patient belonging to the Azerbaijani population studied in this research. Furthermore, it is not known whether 558 of the 584 SNPs identified so far in the CDS portion of the human AGT gene play any clinical role (Table 1). All these facts and uncertainties indicate that the role of genetic factors in the formation of AH is unknown in most cases.

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Effects of dihydropyrimidinase-related protein 2 on anxiety level in rats

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The article concerns studies on molecular mechanisms of anxiety. The studies were carried out on Wistar male rats, through the application of an open field test and an elevated plus-maze model. On the audiogenic stress model, stress-resistant rats were chosen for the following studies. The animals were divided into 3 groups: 1) intact group, 2) control group – inactive dihydropyrimidinase related protein 2 (DRP2), and 3) experimental group – DRP2. Preparations (1 mg/mL, 10 μL) were administered into the brain lateral ventricle of etherized rats 24 h prior to behavioral studies. The results of the studies carried out on the open field test revealed a decline in the number of rearings ($p<0.01$), the total duration of grooming ($p<0.001$), the number of crossed squares ($p<0.01$), and an upregulation of the total duration of freezing in the experimental group ($p<0.01$) relative to the control animals. The results obtained in the elevated plus-maze model showed a sharp decline in the number of rearings ($p<0.001$), total duration of grooming ($p<0.001$), a number of crossed squares ($p<0.001$), a three-fold increase of total duration of freezing time ($p<0.001$) in the closed branch and a prominent, more than four-time decrease in the number of hangings in the experimental animals ($p<0.001$) relative to the control ones. A general conclusion concerning the anxiogenic effects of intra-cerebral administration of DRP2 on animal behavior is put forward.

Keywords: *Male Wistar rats, dihydropyrimidinase related protein 2, open field test, elevated plus-maze, anxiogenic effect*

INTRODUCTION

Nowadays, the problem of the underlying molecular mechanisms of anxiety is still far from its complete clarity. Along with this, it should be noted that this problem has both fundamental and practical importance – evaluation of the emotional status of airport operators and the fatigue of workers on factory conveyor lines. Earlier, we revealed changes in the level of dihydropyrimidinase-related protein 2 (DRP2) in the platelets and saliva and natural anti-DRP2 autoantibodies (Avrameas, 1991; Poletayev, 1995) in the serum of the patients, feeling anxiety on the day of their surgery and having increased levels of the stress hormone cortisol in their serum (Guliyeva, Mekhtiev, 2023). DRP2 is a serotonin-modulating protein known to facilitate axonal outgrowth of the brain neurons (Goshima et al., 1995; Inagaki et al., 2001). However, additional studies are required to clarify the character of such changes in terms of whether they reflect an organism's adaptation to stressful conditions or, conversely, if they are indicative of processes of disadaptation or exhaustion of internal resources. The goal of the present study was to reveal the effect of intra-cerebral administration of DRP2 on the level of anxiety in two behavioral models on rats.

MATERIALS AND METHODS

DRP2 was purified from cow brains through the application of biochemical procedures as

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described earlier (Gaisina et al., 2022). The brains were homogenized in the extracting buffer containing 0.05 M phosphate buffer (pH 7.2), 0.3 M NaCl, 5 mM EDTA and 0.1% Triton X-100 in a volume ratio of tissue and buffer as 1:4. The main stages of fractionations were as follows: 1) protein partial precipitation by ammonium sulfate under the final concentration of 40%, 2) gel-chromatography on the column (3 x 60 cm) of Sephadex G-150, 3) exposure to the effect of 40 mM deionized EDTA throughout the night on the end-to-end shaker, 4) mixture of proteins with EDTA was subjected to isoelectric focusing on a flat-bed gel "Ultrogel" (LKB, Sweden) with the application of ampholines of narrow pH range (pH 4-6; LKB, Sweden). After ending isoelectric focusing, 1 cm width gel strips were collected, pH values were measured in each gel strip and the fraction with a pH value that was equal to the *pI* value of DRP2, was eluted from the gel and analyzed in SDS electrophoresis with protein standards. The process of fractionation and selection of the immune-positive protein fractions was realized under the screening control by the indirect ELISA-test with the application of anti-SMAP polyclonal immunoglobulins (Gaisina et al., 2022).

The behavioral studies were carried out on Wistar male rats of 180-220 g body mass. First, the animals were subjected to audiogenic stress of 90-120 dB strength and only stress-resistant animals were selected for conducting the following series of studies. All stress-resistant rats were divided into 2 groups: 1) intact group, 2) control group – administration of inactive DRP2 (water bath at 60°C, 35 min), and 2) experimental group – administration of active DRP2. The preparations were administered into the brain left lateral ventricle of the anesthetized rats (natrium etaminali 6 mg to 100 g of body mass) at a dose of 1 mg/mL and volume 10 μL, in buffered saline (pH 7.3), at a low speed for 1 min. Behavioral experiments on both models were undertaken 24 h after the administration of the preparation.

The first series of experiments were carried out on the open-field model. This model allows evaluation of both exploratory activity and the anxiety level of the rats. The animals were placed at the center of the experimental box, one at a time, for 1 min under dark conditions and thereafter for 3 min under illuminated conditions (lamp of 150 W power hung at a height of 1 m beyond the floor of the experimental box). The total duration of grooming and freezing acts and the number of rearing acts and crossed squares separately for dark and illuminated conditions were measured.

The second series of experiments was carried out on the elevated plus-maze. This model is a worldwide-accepted model for measuring anxiety levels in rats. The animals, one at a time, were put in the center of the maze and the total duration of freezing and grooming and the numbers of rearing, hanging down, and crossing squares in the closed and open arms of the maze during the 5-minute test were fixed. The average values were calculated for each group and analyzed using the Student's t-criterion.

RESULTS AND DISCUSSION

The results of the experiments undertaken on the open field test demonstrated significant changes in behavioral indices in the experimental group relative to the control group. In particular, under dark conditions, the number of rearings of the experimental animals was less than that of the control ones (1.1±0.5 vs. 2.3±0.2, $p<0.05$), the total freezing time was much higher than that of the controls (18.4±6 sec vs. 0 sec, $p<0.01$) and the number of crossed squares of the experimental group was three times lower than the control values (13.3±1.7 vs. 48.7±2.7, $p<0.001$). Moreover, under the illuminated conditions in the open field test, the differences between the behavioral indices of the experimental and control groups were much sharper than those under dark conditions. In particular, the number of rearing acts was about 4 times lower than in the control group (1.6±0.6 vs. 5.8±1.3, $p<0.01$; Fig.1), total duration of grooming acts was more than three times lower than the values of the control animals (16.7±6.4 sec vs. 57.3±2.9 sec, $p<0.001$; Fig.2), total time of freezing exceeded two times this level of the control rats (127±17.4 sec vs. 64.3±6.6 sec, $p<0.01$; Fig.3), and a number of crossed squares in the experimental group was drastically lower than this index in the control group (29.9±6.9 vs. 49.9±2.3, $p<0.05$; Fig.4).

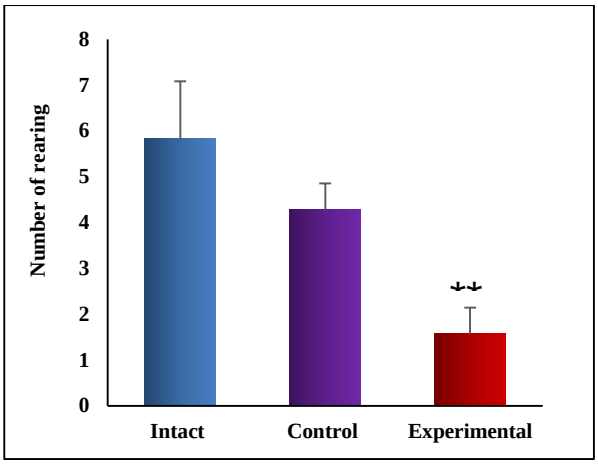

Fig. 1. The number of rearings of the rats in the open field test under illuminated conditions.
** - p<0.01.

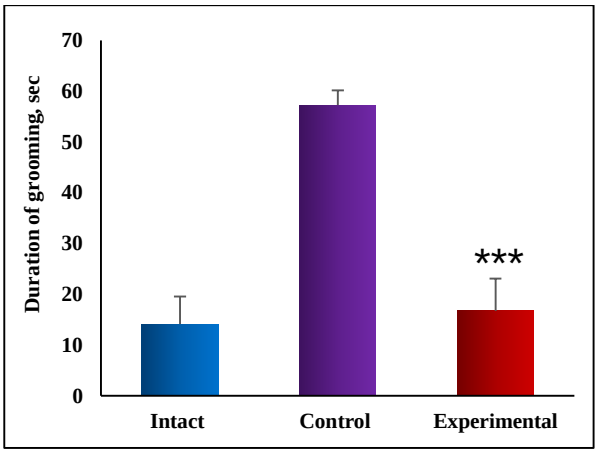

Fig. 2. The total duration of grooming acts in the open field test under illuminated conditions.
*** - p<0.001.

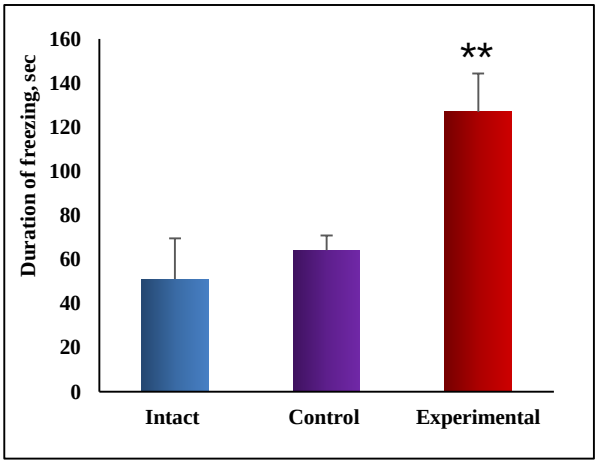

Fig. 3. The total duration of freezing in the open field test under illuminated conditions.
** - p<0.01.

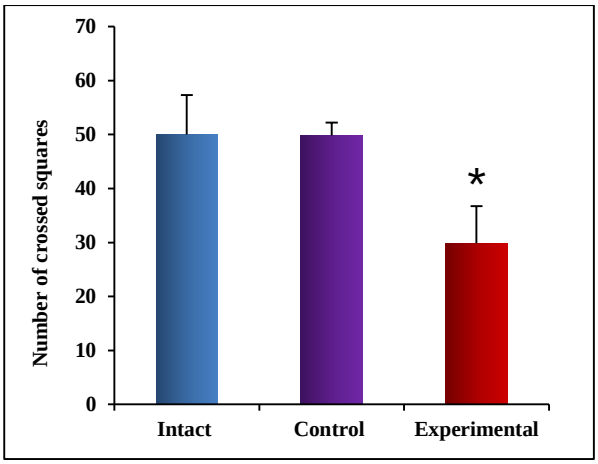

Fig. 4. The number of crossed squares in the open field test under illuminated conditions.
* - p<0.05.

The results of the experiments in the elevated plus-maze model as well revealed significant changes in behavior under the effect of DRP2 intracerebral administration. In particular, in the animals of the experimental group, a sharp decline in the number of rearing acts (1.3±0.5 vs. 13.7±0.6, p<0.001; Fig.5), of the total duration of grooming acts (2.5±1.7 sec vs. 37.3±3.5 sec, p<0.001; Fig.6), of the number of crossed squares (6.2±1.5 vs. 27.7±1.0, p<0.001; Fig.7) in the closed branch of the elevated plus-maze relative to the control values were observed. At the same time, a three-fold increase in the total duration of freezing time in the closed arms in the experimental group relative to the control group (226.7±14 sec vs. 66.3±2.8 sec, p<0.001; Fig.8) was noted.

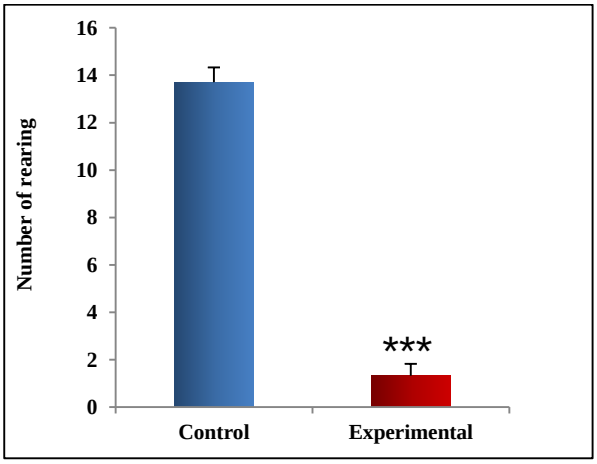

Fig. 5. The number of rearings of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

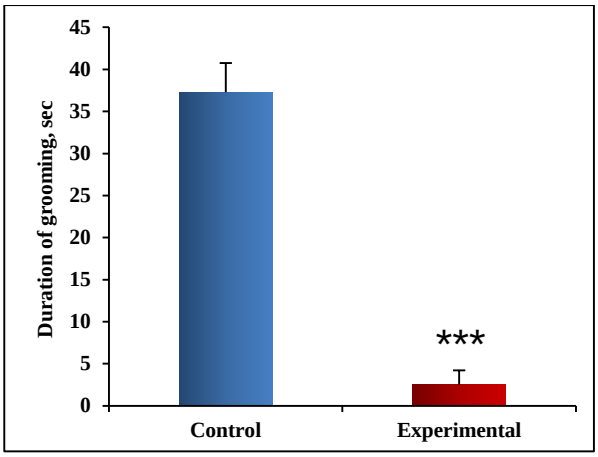

Fig. 6. Total duration of grooming acts of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

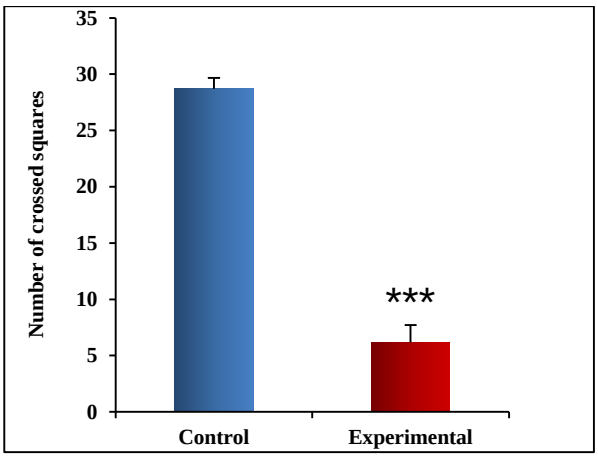

Fig. 7. The number of crossed squares of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

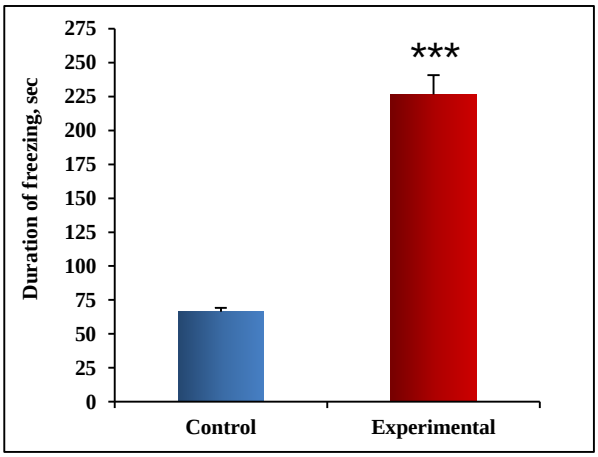

Fig. 8. The total duration of freezing acts of the rats in the closed arms of the elevated plus-maze model.
*** - p<0.001.

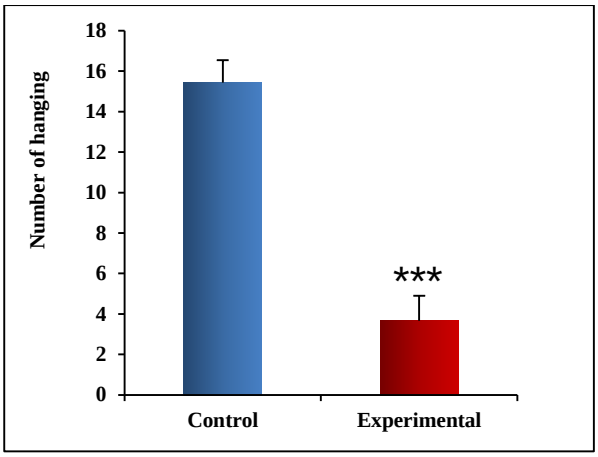

Fig. 9. The number of hangings of the rats in the elevated plus-maze model.
*** - p<0.001.

Furthermore, a number of hangings drastically declined more than four times in the experimental animals relative to the control ones (3.6±1.2 vs. 15.4±1.1, $p<0.001$; Fig.9). There was no difference in the number of crossed squares between the experimental and control groups (4.7±0.2 vs. 5.6±0.5, $p>0.05$).

On the whole, the results obtained from the studies on the rats in two behavioral models designed for analysis of excitation level allowed to reveal significant changes in animal behavior under the effects of intra-cerebral administration of DRP2. Both the results of the open field test – the decline of the number of rearing acts, the total duration of grooming, the number of crossed squares, and the upregulation of the total duration of freezing in the experimental group – and the results of elevated plus-maze model – the sharp decline of a number of rearing acts, of the total duration of grooming acts, of a number of crossed squares, a three-fold increase of a number of total duration of freezing time in the closed branch and prominent, more than four times decrease of the number of hanging in the experimental animals relative to the control ones – give grounds to coming to a general conclusion concerning anxiogenic effects of intracerebral administration of DRP2 on animal behavior.

In terms of the results obtained on rat models of behavioral anxiety, the results by analyzing blood and saliva samples from patients experiencing anxiety on the day of surgery become understandable **(**Guliyeva, Mekhtiev,

2023). The upregulation of DRP2 in the platelets of the excited persons reflects its upregulation in their brain cortex (Collins et al., 2013; Elliott, Kent, 1989), whereas the upregulation of natural anti-DRP2 autoantibodies in their blood serum, according to our own data (Hasanova, 2022), indicates a relative upregulation of DRP2 in subcortical structures. Based on the results we got on the fish (Mekhtiev et al., 2017) and on the rats (Allahverdiyeva et al., 2019), upregulation of DRP2 in the brain structures and generally throughout the body underlies engagement of the adaptation processes, promoting mitigation of the negative effects of adverse factors of different origins on the organism.

CONCLUSION

The results obtained both from the open field test and the elevated plus maze indicate the anxiogenic effects of DRP2 on rat behavior. These results strongly coincide with the results obtained earlier on the patients feeling anxiety on the day of their surgery.

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Influence of the parental forms on the storage ability of the newly evolved grape varieties

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Grapes are an important source of various vitamins and minerals, and cold storage is a way to prolong their consumption period. In Azerbaijan, there are about 600 varieties, the majority of them being table ones, and it is necessary to determine the varieties most suitable for long-term storage. As objects of study, we chose two new hybrid varieties created at the Azerbaijani Institute of Viticulture and Wine-making. The grapes were stored at 0...-1°C temperature and relative humidity of 95% for three months. To prevent decay, we used weekly fumigation with sulphur anhydride. In the Azeri hybrid variety, the outcome of standard production after storage was 72%. So, this variety appeared promising for further studies to determine the optimal harvest time and clarify storage technologies.

Keywords: *Variety, storage ability, type of losses, perspective*

INTRODUCTION

Azerbaijan is among the most perspective regions for the development of viticulture in different use directions. The favourable location, various natural conditions in connection with mountainous-plane orography, and considerable diversity of the climatic and soil conditions of viticulture regions make it possible to grow here the grapes of all ripening periods for processing and for local consumption, long-term storage and transportation to industrial centres, as well as drying (Panahov et al., 2010; Sharifov, 2013). Due to their nutrition and diet characteristics, grapes positively affect human health (Restani et al., 2016; di Lorenzo et al., 2015, 2016). However, as a seasonal product, the grape`s shelf life is restricted to 3 to 4 months. One of the ways to extend the period of fresh grapes consumption is by organizing its cold storage during the winter season. Storageability means the ability of grape bunches to preserve their appearance and organoleptic features for several months without considerable weight losses and damages caused by phytopathological and physiological diseases. The individual traits of the variety are the most important indicator that provides the success of a grape`s long-term storage (Jeneyev, 1971; Ahmadi and Vafaee, 2023). Not all the best table grape varieties are fit for storage and transportation because of the thinness of their skin. Azerbaijan is the country of origin of the numerous table grape varieties distinguished by good taste, attractive appearance, transportability and storage ability. Also, highly productive introduced varieties with high-quality berries are widespread here. Unfortunately, a relatively low number of varieties are suitable for long-term storage Fully ripened varieties of the late-ripening period, with pulpy and crusty berries and loose bunches, are more appropriate for this purpose, for example in the conditions of Azerbaijan, these

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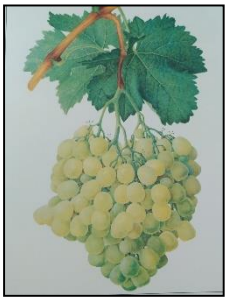
are Aghadayi, Tayfi pink, Gara shany, Tabrizi, Moldova, etc. (Negrul et al., 1973; Asadullayev and Abasova, 2012; de Lorenzis et al., 2015).

At different times, researches were carried out in Azerbaijan on the selection of grape varieties suitable for long-term storage. In this publication, we present the results of cold storage of Azeri (Katta-Kurgan x Tabrizi) and Genjevi (Ag Shany x Bayanshira) varieties created at the Experimental Station in Ganja, west of Azerbaijan Republic.

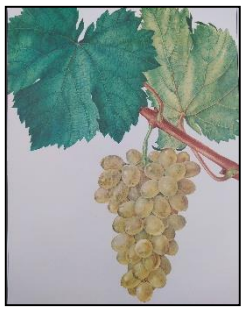
MATERIALS AND METHODS

Below are the short descriptions of the parental forms of the studied varieties.

Ancestors of the **Azeri** variety – Katta Kurgan and Tabrizi.



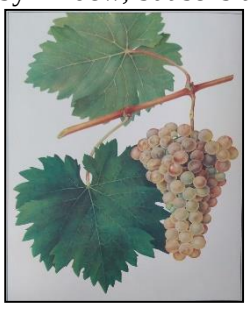
Katta-Kurgan - table grape variety of the late period of ripening. Homeland - Central Asia. It belongs to the eco-geographical group of oriental grape varieties. The leaves are large, fan-shaped, slightly dissected, five-lobed, smooth, glabrous. The flower is functionally feminine. Clusters are large, wide-conical, winged, loose or of medium density, and weigh an average of 400 g. Berries are large, round-oval or obovate, light green. The skin is thick and fragile. The pulp is fleshy, juicy, and tender. The period from the beginning of bud break to consumer maturity of berries is 145 days at a sum of active temperatures of 2950°C. Shoot maturation is good. Bushes vigorous. Productivity is 70-80 c/ha. Relatively resistant to fungal diseases. The frost resistance is weak. The yield is used for fresh consumption and drying.



Tabrizi - Azerbaijani table grape variety of medium ripening. It belongs to the eco-geographical group of oriental table grape varieties. The leaves are large, five-lobed, funnel-shaped, dark green, shiny, slightly reticulate-wrinkled, glabrous below. The type of flower is hermaphroditic. Bunches are medium, conical, and dense. The berries are medium, oval, white, and golden when overripe. The peel is elastic, durable, and pruinose. The pulp is juicy. The period from the beginning of bud break to the full ripening of grapes is 140 days at a sum of active temperatures of 3490°C. Shoot maturity is good. Bushes are vigorous. Productivity is 100-150 c/ha. Variety is slightly damaged by mildew, and non-resistant to oidium, frosts and Lobesia botrana. Highly transportable and of good storage ability. The yield is used for fresh consumption.

Ancestors of the **Genjevi** variety – Ag shany and Bayanshira.

Ag shany - Azerbaijani table grape variety of medium ripening period. It belongs to the eco-geographical group of oriental grape varieties. The period from the beginning of bud break to the full maturity of berries in Azerbaijan is on average 121 days at a sum of active temperatures of 2600°C. Bushes are vigorous. The leaves are large, pentagonal in shape, slightly dissected, with barely prominent lobes, there is no pubescence below. The petiole notch is tightly closed. The lower blades overlap one another very deeply. The flower is functionally feminine. The clusters are medium or large, wide-conical or cylindrical-conical, sometimes with developed lobes, branched at the base, density varies depending on pollination. The berries are large, oval, somewhat asymmetrical, whitish-yellow, golden yellow when fully ripe, and light brown on the sunny side. Shoot maturation is good. Productivity is 50-90 c/ha, with irrigation 200-400 c/ha. The variety is drought tolerant. Ag shany is slightly damaged by mildew, but sensitive to oidium.



Bayanshira – Azerbaijani wine grape variety of late ripening period. It belongs to the eco-geographical group of oriental grape varieties. The leaves are large, rounded, five-lobed, medium dissected, folded or funnel-shaped, grooved,

glabrous below. The petiolate notch is lyre-shaped with a sharp bottom or closed with a lumen. The flower is hermaphroditic. Clusters are medium or large, loose, cylindrical, dense. The berries are medium, almost large, round, greenish-yellow, with brown spots when overripe. The peel is of medium thickness, covered with a wax coating. The pulp is juicy. The period from the beginning of bud break to the harvesting maturity of grapes is 165 days at a sum of active temperatures of 3500°C. Shoot maturation is good. Bushes are vigorous. Productivity is 120-200 (when irrigated - up to 350 c/ha). The grape variety Bayanshira is moderately resistant to mildew, oidium, sensitive to frost and drought, grey mould and phylloxera.

RESULTS AND DISCUSSION

Below we present the ampelographic descriptions of studied grape varieties using Multi-crop passport descriptors (MCPD) for Grapevine (Alercia et al., 2015).

Table 1. Description of the Azeri grape variety

	Azeri B (AZE007)		
	Multi Crop Passport Descriptor Data		
	Color of berry skin	green	
	Variety name	Azeri	
	Genus, Species	VITIS VINIFERA LINNÉ	
	Country of origin of the variety	Azerbaijan	
	Use (crop name)	WINE GRAPE	
	Holding institution	*Scientific Research Institute of Viticulture and Winemaking of Azerbaijan*	
	Priority descriptor data		
OIV 004	Young shoot: density of prostrate hairs on the shoot tip	3-low	
OIV 051	Young leaf: color of upper side of blade (4th leaf)	2- yellow	
OIV 068	Mature leaf: number of lobes	2- three	
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on the upper side of blade	1- only at the petiolar point	
OIV 076	Mature leaf: shape of teeth	3- both sides convex	
OIV 079	Mature leaf: degree of opening / overlapping of petiole sinus	9- strongly overlapped	
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	1- none or very low	
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade	1- none or very low	
OIV 223	Berry: shape	2-globose or 3- broad ellipsoid	
OIV 225	Berry: color of skin	1- green yellow	
	Bunch / berry descriptor data		
OIV 202	Bunch: length (peduncle excluded)	9-very long (about 170-230 mm)	
OIV 204	Bunch: density	5- medium	
OIV 206	Bunch: length of peduncle of primary bunch	3-short (about 30-40 mm)	
OIV 209	Bunch: number of wings of the primary bunch	2- (1 – 2 wings)	
OIV 220	Berry: length	7- long (about 18-23 mm)	
OIV 221	Berry: width	5 medium (about 17-22 mm)	
OIV 502	Bunch: single bunch weight low	7- high or very high (about 750-860 g)	
OIV 503	Berry: single berry weight	7- high (about 4-5 g)	
	Agronomic features		
OIV 301	Time of bud burst	5- medium	
OIV 303	Time of beginning of berry ripening (veraison)	5- medium	
OIV 351	Vigor of shoot growth	7- strong	
OIV 504	Yield per m²	9- very high	
OIV 505	Sugar content of must	7- high (about 19-20%)	

Table 2. Description of the Genjevi grape variety

	Genjevi B (AZE007)		
Multi Crop Passport Descriptor Data			
	Color of berry skin	green	
	Variety name	Genjevi	
	Genus, Species	VITIS VINIFERA LINNÉ	
	Country of origin of the variety	Azerbaijan	
	Use (crop name)	WINE GRAPE	
	Holding institution	*Scientific Research Institute of Viticulture and Winemaking of Azerbaijan*	
	Priority descriptor data		
OIV 004	Young shoot: density of prostrate hairs on the shoot tip	1-none or very low	
OIV 051	Young leaf: color of upper side of blade (4th leaf)	2- yellow	
OIV 068	Mature leaf: number of lobes	2- three or 3-five	
OIV 070	Mature leaf: area of anthocyanin coloration of main veins on the upper side of blade	1- only at the petiolar point	
OIV 076	Mature leaf: shape of teeth	3-both sides convex	
OIV 079	Mature leaf: degree of opening / overlapping of petiole sinus	5-closed	
OIV 084	Mature leaf: density of prostrate hairs between main veins on lower side of blade	1- none or very low	
OIV 087	Mature leaf: density of erect hairs on main veins on lower side of blade	1- none or very low	
OIV 223	Berry: shape	7- ovoid	
OIV 225	Berry: color of skin	1- green yellow	
	Bunch / berry descriptor data		
OIV 202	Bunch: length (peduncle excluded)	9-very long (about 160-220 mm)	
OIV 204	Bunch: density	3-loose	
OIV 206	Bunch: length of peduncle of primary bunch	5-medium or 7-long (about 40-55 mm)	
OIV 209	Bunch: number of wings of the primary bunch	2- (1 – 2 wings)	
OIV 220	Berry: length	7- long (about 17-21 mm)	
OIV 221	Berry: width	5- medium (about 15-19 mm)	
OIV 502	Bunch: single bunch weight low	5-medium (about 370-430 g)	
OIV 503	Berry: single berry weight	5-medium or 7- high (about 4-6 g)	
	Agronomic features		
OIV 301	Time of bud burst	5- medium	
OIV 303	Time of beginning of berry ripening (veraison)	3-early	
OIV 351	Vigor of shoot growth	7- strong	
OIV 504	Yield per m²	9- very high	
OIV 505	Sugar content of must	7- high (about 18-19%)	

The experimental lot of Azeri and Genjevi grape varieties was put in cold storage in the Tovuz region in the western part of Azerbaijan and stored at 0ºC and relative humidity of 95% for three months. To suppress the activity of epiphyte microflora, weekly fumigation with sulfurous anhydride was applied. Tayfi pink and Tabrizi varieties served as control. The obtained results are shown in Table 3.

Table 3. Yield of standard product and composition of losses after 3 months of storage

Variety	Standard product, %	Losses composition, %		
		rotten	squashed	detached
Azeri	72	69	22	9
Genjevi	60	100		
Tayfi	87	70	30	
Tabrizi	100			

As we can see, the control varieties Tayfi and Tabrizi underwent long-term storage considerably better than the tested hybrids. It can be explained by the presence of worked storage technology for these varieties. At the same time, those created in our institute need the factors affecting the storage ability, such as ripening period, growing and storage conditions, - to be specified. Also, the study of the features defining the storage ability of the variety (transportability, natural weight loss, change of chemical composition and microflora during the storage period, etc.) should continue. Concerning the character of the losses, in the Azeri variety, they comprised mainly of microbiological rot, the amount of which was almost equal to the same indicator of the control variety of Tayfi. Both the paternal forms of this hybrid are distinguished by low transportability, and 22% of losses of the Azeri variety during the storage period consist of squashed berries; however, this is less than that in control. One of the paternal forms – the Tabrizi variety, is presumed to be little tolerant to sulfur dioxide, and during the fumigation, there could occur the destruction of peduncles and tissues of berries in the pedicel area; in the Azeri variety, we observed the considerable drying of the peduncle during storage period that led to 9% of losses because of the detached berries. As for the Genjevi variety, like one of the parental forms – Ag shany appeared to be significantly damaged by pathogenic microflora during the storage period; the losses (40%) entirely consisted of the rotten berries. In addition, by the end of the storage period, the bunches of this variety showed significant browning of the stem.

According to numerous literature sources, the individual characteristics of the variety have the most significant influence on the storage ability of the grapes (Burger et al., 2005; Ejsmentwicz, 2015, Potapenko and Ganich, 2015, 2016). The preliminary results indicate the presence of a visible connection between the physical-mechanical features of the parental forms and the storage ability of the hybrid varieties studied. Judging the outcomes of the studies conducted, we can conclude that the studied hybrid varieties, especially Azeri, paternal forms of which possess the high (Tabrizi) and medium (Katta-Kurgan) storage ability, deserve to be subsequently studied for working out of the optimal storage technology.

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Estimation of determinate varieties of tomatoes (*Solanum lycopersicum* L.) in irrigating conditions of the Absheron region

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The research conducted at Genetics Resources Institute focused on the cultivation of determinate tomato varieties in the irrigating conditions of the Absheron Region. In this area, tomato growers encounter challenges related to water scarcity and mismanagement, which significantly impact their income and the local ecosystem. Given the vital role of water in tomato cultivation, the study investigated the impact of irrigation on the quality of determinate tomatoes intended for processing. The experiment, conducted between 2017 and 2018 in Absheron's experimental fields, involved treating different determinate tomato varieties with various irrigation regimes, which varied based on water quantity and the interval between irrigations. Eight standard varieties with varying stem lengths were selected from the gene pool of determinate tomatoes for the experiment. The primary objectives were to analyze the morphological and biological traits of these varieties and identify potential donors with economically beneficial characteristics for future tomato breeding efforts. Additionally, the study aimed to determine the optimal irrigation schedule for determinate tomato varieties to strike a balance between yield and water usage efficiency. Two experiments were carried out, each monitoring variables related to determinate tomato production, including plant height, stem diameter, fruits per plant and overall yield. The findings indicated that the quantity of water applied had a more significant impact on fruit quality and production than the frequency of irrigation.

Keywords**:** *Water dosage, irrigation frequency, tomato quality, tomato production, water use efficiency*

INTRODUCTION

Plant breeders have extensively utilized determinate tomatoes in recent years to enhance yield levels in various cross-pollinated crops (Huseynzade et al., 2020). The process of domesticating and improving crops through breeding has proven highly effective in concentrating allelic variation that imparts useful characteristics for both cultivation and consumption (Osborn, Chad, Elaine, and Carl, 2007). Tomato occupies a special place among vegetable crops. The nutritional properties of fruits, which are used fresh and pickled in the canning industry for the manufacture of ketchup, juice, mashed tomatoes, and other products, contribute to their widespread use. The quality and yield of tomatoes depend on their variety (Albert et al, 2016a). Determinate varieties of tomatoes have a compact bush, which allows long-term inter-row cultivation. They can be grown with seedlings 2 times thicker than ordinary varieties, and therefore the cost of growing seedlings per 1 ha is 2 times cheaper. In the determinate varieties, the fruit is in less contact with the soil than in ordinary varieties (Avdeev, 2006). In connection with the value of determinate varieties, the study of their biological properties is of great importance. In the selection and collection of the gene pool of tomatoes, we selected determinate samples that differ in stem length. It is of interest to study their differences in other biological and economic characteristics.

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The primary aim of the experiment was to examine the morphological and biological attributes of determinate tomato varieties, particularly focusing on those grown under irrigating conditions in the Absheron Region.

This study also aimed to identify individuals possessing economically beneficial traits, thus contributing to the broader goal of utilizing these selected varieties in future tomato breeding programs.

Many studies have assessed plant responses to different watering regimes in several species and shown the negative impact of water shortages on plant growth and yield. Reviews of the different morphological, physiological, and molecular changes induced by water limitation are available (Chaves et al., 2003; Hirayama and Shinozaki, 2010; Farooq et al., 2012; Silva et al., 2013).

Irrigation mismanagement affects water storage in reservoirs (Irmak et al., 2016) and can be improved if the water dose and frequency of irrigation is correctly determined (Wang et al., 2006).

MATERIALS AND METHODS

The experiment took place in the experimental fields of the Absheron region at the Genetic Resources Institute during 2017-2018. Determinate tomatoes were initially planted in the greenhouse in the first decade of March, and then transplanted as seedlings into the open experimental field in the last decade of April. Seedling care included watering, weeding, and loosening of the soil. Plants were fertilized with mineral fertilizers at a rate of N10P20K15 per 10 liters of water, which were applied to plants in the open area, followed by irrigation. Tomatoes were planted using a scheme of 150 x 35 cm spacing. Throughout the growing season, three inter-row cultivations were conducted at a rate of 210-270 m^3/ha, and weeding was carried out three times manually. Additionally, nine irrigation sessions were performed. Eight types of determinate tomatoes were studied: Volqaqrad-5\95, Ordubad, Krosnodar, Ilkin, Shahin, Varonej, Utro and Azerbaijan.

Geography and climate. Azerbaijan, positioned in SW Asia or the far SE of Europe, covers an area of 86,600 km^2 between 38º25’ and 41º5’N and 50º-50º51’E, bordered by the Caspian Sea to the east. In the Greater Caucasus mountains, temperatures average 14-16º C at 2,000 m elevation and remain above 8-10ºC at 3,000 m.

Absheron, partly on a peninsula and partly inland, lies along the west coast of the Caspian Sea of Baku.

Absheron soils are typically influenced by the semi-desert to dry-desert climate of the area and are characterized by their arid conditions. Absheron soils may vary but often exhibit characteristics such as low organic matter content, high salinity levels, and poor fertility. Due to these conditions, agriculture in the region often requires careful irrigation and soil management practices to support plant growth. Additionally, the presence of salt lakes in the area can contribute to soil salinization, further impacting agricultural productivity.

Experimental design. The present study is focused on finding the optimal irrigation schedule for determinate tomato crops that balances yield and quality. Thus, several doses of water and the frequency of irrigation were evaluated. In the first experiment, two watering frequencies were controlled: two waterings per day (F1) and one watering per day (F2), as well as four water doses: 80% (L1), 100% (L2), 120% (L3) and 140% (L4) dose-control. In the second experiment, four watering frequencies were evaluated: two waterings per day (F1), one watering per day (F2), one watering every two days (F3), and one watering every three days (F4), as well as two doses of water (the best of the first experiment): 100% (L1) and 120% (L2). In the first experiment, a factorial arrangement (2 × 4) was performed on divided plots in a fully randomized four-replication block design. In the second experiment, a factorial design (2 × 4) was performed in a completely randomized block design with four repetitions.

Measurements. The study tracked field agronomic variables, fruit quality parameters, and water use efficiency. Agronomic and fruit quality variables were measured over a sampling area that was representative of the site; thus, in the first and second experiments, 10 and 12 plants were selected (located in the center of the plot), respectively.

Agronomic Variables. The selected agronomic variables were plant height, stem diameter, number of fruits per plant, and productivity. These variables were measured in all plants in the sampling area. A measuring tape was used for the height of the plant, and measurements were made from under the trunk to the entry point of the last truss. At the end of each experiment, the total and marketable products are calculated. In terms of fruit weight and taking into account the parameters set by the Ibarra Wholesale Market (Imbabura Province), the fruits were divided into four categories, ie <70 g (very small fruit), 70-100 g (small fruit), 100-150 g (medium size fruit) and> 150 g (large fruit). Very small, deformed and cracked fruits were considered unsold.

Quality Parameters. Quality indicators were monitored in two and three samples for each experimental site, and each sample was analyzed separately. In the first experiment, samples were taken 119 and 130 days after sowing, and in the second experiment, 135 and 160 days after sowing. For each sample, three fruits were selected (same in size, degree of maturity, without external defects).

RESULTS AND DISCUSSION

Determination of tomato production and quality. Table 1 describes the outcomes from the ANOVA and Duncan's Multiple Range test tests of the first experiment. The effect of water dose on plant height was more considerable than the effect of irrigation frequency in the first experiment. The irrigation dose applied (140%) and the 120% resulted in plants with the greatest heights: 129.90 and 116.43 cm, respectively. The smallest height corresponded to 102.59. In the second experiment, the effect of water dose and irrigation density was not significant.

All parameters are given in charts (Fig. 1-6). The first figure illustrates how different water doses affect the plant height of determinate tomato plants. It shows the trend of plant height increasing or decreasing with varying water doses.

The second figure depicts the correlation between water doses and the number of fruits produced per determinate tomato plant. It shows how changes in water doses influence the fruit yield per plant.

Table 1. Analysis of variance and Duncan's multiple range test of the average plant height, stem diameter, fruits per plant, total yield, marketable yield, total water use efficiency and marketable water use regulation for the first experiment

Factor	Plant height (cm)	Stem diameter (mm)	Fruits Plant (-)	Total Yield (kg plant^{-1})	Marketable yield (kg plant^{-1})	Total WUE (kg m^{-3})	Marketable WUE (kg m^{-3})
Irrigation frequency							
F1	106.08	13.56	47.63	11.05	10.08	49.83	46.50
F2	105.33	13.73	47.50	11.81	11.08	54.39	51.48
Water doses							
L1-80%	102.59	10.44	47.39	10.92	8.89	57.32	53.68
L2-100%	114.13	10.62	48.66	12.00	11.12	53.26	50.32
L3-120%	116.43	12.18	48.53	12.95	12.15	49.89	45.56
L4-140 %	129.90	13.35	49.79	14.27	13.36	45.97	43.81
Anova							
F	ns	Ns	ns	Ns	ns	ns	ns
L	**	***	ns	***	***	***	*
F × L	ns	Ns	ns	Ns	ns	ns	ns

F and L represent irrigation frequency and water height, respectively. F1: one irrigation per day; F2: two irrigations per day; L1: 80%; L2: 100%; L3: 120% ; L4: 140% ETc ; WUE: water use efficiency;*: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$; ns: no significant at $p < 0.05$

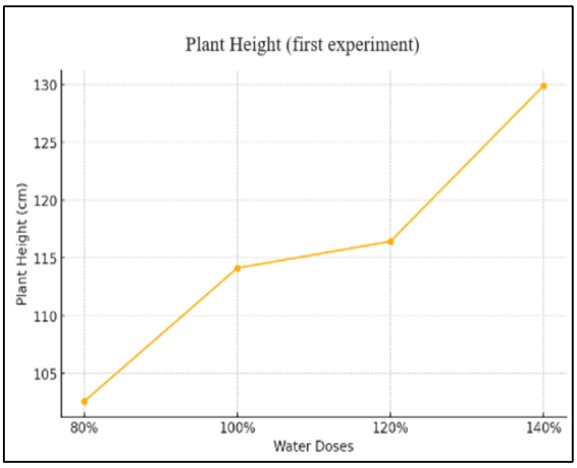

Fig.1. The relationship between water doses and plant height

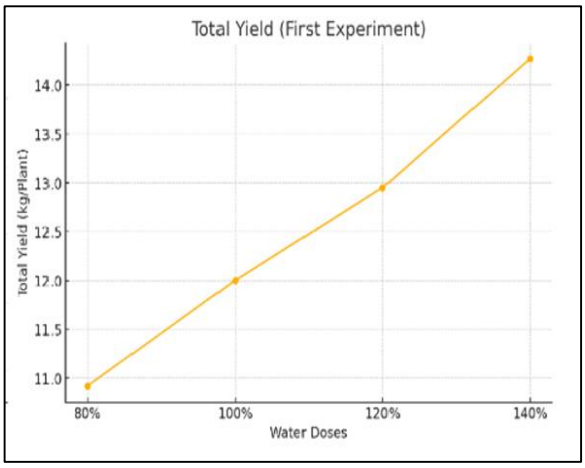

Fig. 4. The relationship between water doses and total yield

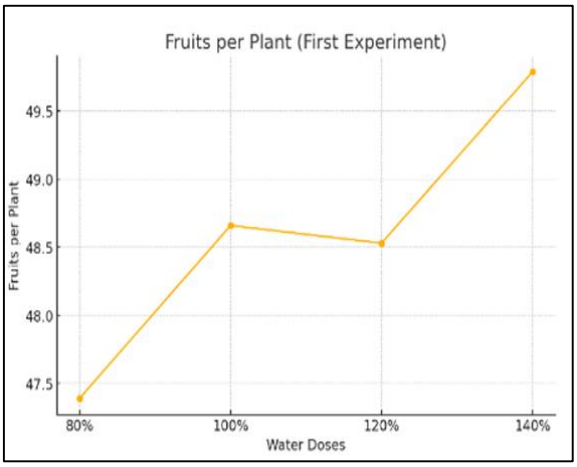

Fig. 2. The relationship between water doses and fruits per plant

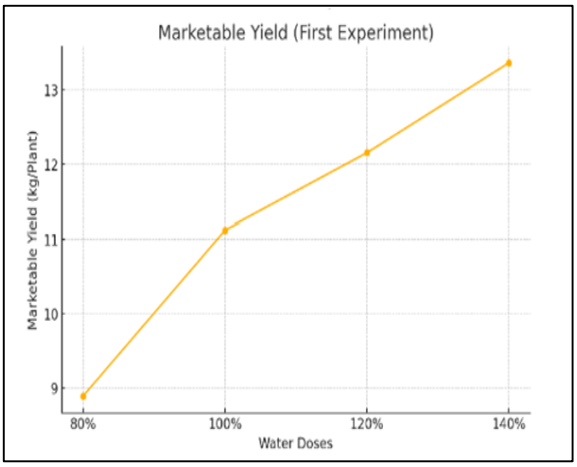

Fig. 5. The relationship between water doses and marketable yield

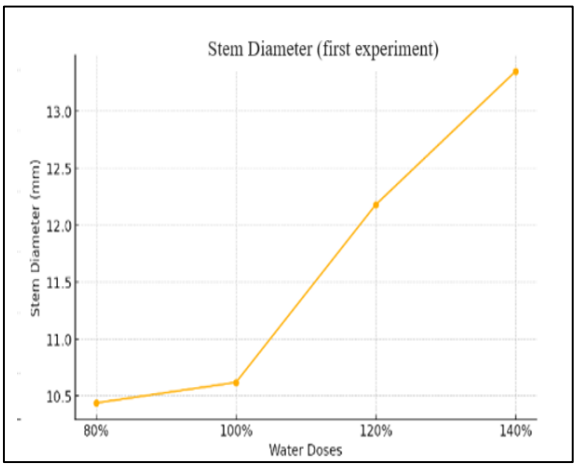

Fig. 3. The relationship between water doses and stem diameter

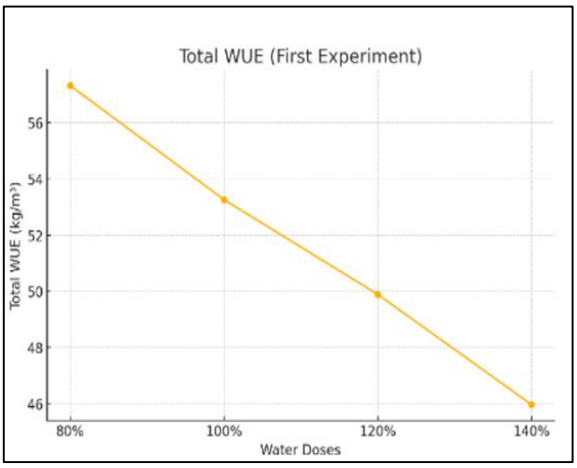

Fig. 6. The relationship between water doses and marketable yield

The third figure demonstrates the impact of different water doses on the stem diameter of determinate tomato plants. It visualizes how water availability affects the thickness of the plant stems. The forth figure shows the relationship between water doses and the total yield of determinate tomatoes. It presents how variations in water doses contribute to changes in the overall yield of the tomato plants.

The fifth figure displays how different water doses influence the marketable yield of determinate tomatoes. It highlights the relationship between water availability and the yield of tomatoes suitable for the market.

The sixth figure likely provides additional information or a different perspective on the relationship between water doses and marketable yield compared to the fifth figure. It may offer insights into the marketable yield under varying irrigation conditions.

The effect of water dosage on stem diameter was prominent, but the effect of irrigation frequency was not noticeable in the first experiment. The water doses L4 and L3 resulted in the highest stem diameters, whereas the L1 dosage produced the smallest diameter (10.44 mm). In the second experiment, the effect of both water dose and irrigation frequency was significant. Thus, the L3 resulted in the highest stem diameter, and it coincides with the first experiment.

So, one and two irrigations per day presented the highest stem diameters, whereas one irrigation every three days showed the lowest. Similar results were noticed in the first experiment.

Number of Fruits per Plant. As provided in Tables 1 and 2, the effect of water dosage and irrigation density on the number of fruits per plant was not important in both experiments.

Yield. In the first experiment, no significant disparities in irrigation frequencies were observed, but they were detected in the second experiment. Both total and marketable yields raised at irrigation frequency increased. In both experiments, the highest water doses resulted in the highest yields (Tables 1, 2). In the first experiment, irrigation frequency did not have an effect on yield. In the second experiment, the frequencies of one and two irrigations per day resulted in a greater total yield and together with one irrigation every three days, it resulted in the highest marketable yield.

Water Use Efficiency. In the first experiment, no significant differences in irrigation frequencies were noted, but water doses significantly differed. In the second experiment, no significant differences were detected in water doses, but irrigation frequencies differed.

Table 2. Analysis of variance and Duncan's multiple range test of the average plant height, stem diameter, fruits per plant, total yield, marketable yield, total water use efficiency and marketable water use efficiency after the second experiment

Factor	Plant height (cm) (135)	Plant height (cm) (after 160)	Stem diameter (mm)	Fruits plant (-)	Total Yield (kg plant^{-1})	Marketable yield (kg plant^{-1})	Total WUE (kg m^{-3})	Marketable WUE (kg m^{-3})
Water doses								
L1	108.39	101.39	10.93	85.44	6.99	6.36	44.59	36.51
L2	108.52	102.29	12.44	87.31	8.25	7.68	44.46	37.92
Irrigation frequency								
F1	107.83	104.55	12.56	97.00	9.32	7.77	47.69	40.55
F2	109.00	103.05	12.16	95.75	8.68	7.13	44.73	47.71
F3	107.95	98.74	12.37	97.25	8.08	6.34	42.22	44.26
F4	109.23	99.14	10.63	95.50	8.39	6.84	43.45	46.34
ANOVA								
L	ns	ns	***	Ns	***	***	ns	ns
F	ns	ns	**	Ns	*	*	*	*
L × F	ns	ns	ns	Ns	ns	ns	ns	ns

L and F represent water height and irrigation frequency, respectively. L1: 100% ETc; L2: 120% ETc; F1: two irrigations per day; F2: one irrigation per day; F3: one irrigation every two days; F4: one irrigation every three days; WUE: water use efficiency; *: significant at $p < 0.05$; **: significant at $p < 0.01$; ***: significant at $p < 0.001$; ns: no significant at $p < 0.05$.

As shown in Table 1, the 80% and 100% doses resulted in the highest WUE and 40%, the lowest in the first experiment. Overall, two and one irrigations per day achieved the highest total WUE in the second experiment. Likewise, the highest marketable WUE was attained for the frequencies: one and two irrigations per day, and one irrigation every three days.

Discussions. This study examined the impact of varying water doses and irrigation frequencies on crop production, crop quality, and water use efficiency. The results showed that reduced water doses led to decreases in plant height, stem diameter, and tomato yield. These findings are consistent with previous studies by Biel et al. (2021) and Agbna et al. (2017), which reported a reduction in vegetative growth and fruit yield under deficit irrigation conditions. Similarly, a 2020 study found that water stress negatively affected plant height and stem diameter. However, neither water doses nor irrigation frequencies influenced the number of fruits per plant. This aligns with Shabbir et al. (2020), who observed a consistent number of fruits per plant at both 100% and 75% irrigation levels, although other research indicated that water dose can affect fruit number. Tomato plants are highly sensitive to water stress during flowering and fruiting, which can lead to flower abortion and reduced fruit numbers (Zegbe et al., 2006). Despite this, our study did not observe a decrease in fruit number due to water stress.

Water doses had a significant effect on tomato yield, corroborating previous studies that found small water doses negatively impact fruit yield (Giuliani et al., 2017; Liu et al., 2019). Sezen et al. (2010) reported the highest tomato yield with the highest water dosage (150%), which is consistent with our findings. Conversely, Neto et al. (2017) found that weekly irrigation frequencies resulted in the highest yields, while daily irrigation led to the lowest yields. Similarly, Fara et al. (2019) noted that frequent irrigations increased nutrient leaching from the root zone and reduced root system development.

Our study showed only slight differences between total and marketable yields. In terms of marketable yield, one irrigation every three days and one to two irrigations per day produced similar results. However, for total yield, one irrigation per day and every two days resulted in the highest values. The best water use efficiency (WUE) was observed with the lowest irrigation dose, aligning with findings from Wang et al. (2017), Silva et al. (2019), and Mendoça et al. (2020). The highest WUE was achieved with the highest irrigation frequency, which is in line with Oke et al. (2020), who noted that increased irrigation frequency boosts fruit number and yield, thereby improving WUE. However, in our study, the number of fruits per plant was not influenced by either irrigation frequency or water dose. The highest yields and WUE were observed with two irrigations per day, which also produced over 10% more large-sized fruits compared to other frequencies. Conversely, the lowest WUE was recorded with irrigation every other day, resulting in a higher percentage (10% to 21%) of unmarketable fruits.

CONCLUSIONS

Daily irrigation schedules are advisable to better adjust to the variable climatic conditions in the region.

Water scarcity and mismanagement significantly impact tomato growers' income and the local ecosystem. The quantity of water applied has a more significant impact on fruit quality and production than the frequency of irrigation. Water doses significantly affect tomato yield, with small water doses negatively impacting fruit yield. The highest marketable water use efficiency was achieved with one or two irrigations per day and one irrigation every three days. The highest total water use efficiency was observed with two irrigations per day (L1 and L2) in the second experiment. Water dose and irrigation frequency play crucial roles in determining plant height, stem diameter, and tomato yield. Frequent irrigation may result in higher nutrient leaching and less developed root systems. The number of fruits per plant was not significantly affected by irrigation frequency or water dose in the study.

Optimal irrigation schedules can help strike a balance between yield and water usage efficiency in determining tomato cultivation. These conclusions highlight the importance of efficient irrigation practices and water management

strategies in enhancing tomato production and quality in regions facing water scarcity challenges. Both total and marketable yields showed a proportional relationship with water doses.

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Effect of high temperature on mulberry silkworm viability in the embryonic stage of development

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This research paper presents the preliminary results of experiments on the study of the resistance of mulberry silkworm embryos to critical temperatures during egg incubation. The response of breeding lines to critical temperatures at +34ºC was found to be different, according to the study. Thus, in some families of Lines 31 and 301, the revitalization of eggs at high temperatures was from 72.0 % to 84.0 %. However, the number of such resistant breeding families is not large. The different responses of breeding lines to critical temperatures indicate that mulberry silkworm genotypes are resistant to this unfavorable factor to varying degrees.

***Keywords:** Mulberry silkworm, egg, selection, embryo, caterpillars*

INTRODUCTION

The yield of mulberry silkworm cocoons depends on the number of healthy worms and the average weight of the cocoon. Naturally, the preservation of the maximum caterpillar population is dependent on the viability of caterpillars. Studies of mulberry silkworm breeding have shown that one-sided selection aimed at increasing the silkiness of cocoons leads to a decrease in egg revitalization and caterpillar viability (Zhang et al., 2022). If a breeder regularly selects cocoon silkiness, this breeding population will show a dramatic deterioration in caterpillar viability (Umarov et al., 2020). However, breeders face two main problems: increasing the viability of caterpillars and maintaining a high yield and cocoon quality. These issues are among the most difficult problems in mulberry silkworm breeding; breeders try to solve these problems by relying on different methods. As it is known, there are a number of breeds and breeding lines at the Silk Research Institute (Uzbekistan). Some of them have good characteristics of cocoon weight or silkiness, while others have a high potential for caterpillar viability. However, it is difficult to identify breeds with good silkiness and strong caterpillars that are resistant to different climatic conditions.

The seasonal differences in the environmental components considerably affect the genotypic expression in the form of phenotypic output of silkworm crops, such as cocoon weight, shell weight, and cocoon shell ratio (Rahmathulla, 2012). The result indicated a higher level of thiobarbituric acid reactive substances (TBARS, as an index of lipid peroxidation) and total hydroperoxides in the male pupae exposed to high temperatures (40±1°C) (Umarov and Nasirillaev, 2023).

The research design used was a complete randomized design (CRD) with four treatments and five replications, each consisting of 20 larvae. The treatment consists of administering heat shock at temperatures of 34, 38, and 42°C, and without heat shock (ambient temperature). Heat shock was conducted in the 4th instar for 3 hours. The research results showed that heat shock could increase the percentage of mortality, accelerate the larval stage, and reduce larval weight by

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lowering growth; consumption, and digestibility (Tanjung et al., 2017).

The biological architecture determining post-embryonic development and traits is well programmed during the process of morphogenetic movements and organogenesis in the embryo. However, changes in the environmental temperature for a few hours, which is uncommon, affecting the embryo development, protein expression, and hatching of larvae in the Eri silkworm (*Samia cynthia ricini*) remain enigmatic. Hence, for the first time, the eggs of new Eri silkworm breed C2 were exposed to heat shock (HS) temperatures of 35°C, 40°C, and 45°C for 2 hours, not only to measure heat sensitivity but also to uncover differential expression of proteins at a different age of the embryo (Punyavathi et al., 2022).

Therefore, in summer and fall, small, silky-thin cocoons are obtained. At the same time, it is important to try and select breeds and hybrids that are resistant to hot weather conditions and create new breeds and hybrids suitable for the summer and fall seasons. Extreme temperature and humidity conditions resulted in the prolongation of eclosion in silkworm, *Bombyx mori* L. it is extending for two consecutive days. A reduction in eclosion (%) in bivoltine bread NB4D2 was recorded (Reddy et al., 2002). On the other hand, PM (a pure breed of silkworm) and PM × NB4D2 (a hybrid of silkworm) confined their eclosion to a single day (Umarov and Nasirillaev, 2023).

This is related to the aforementioned scientific problem (Akhmedov, 1998), found in their experiments the effect of contrasting air temperature and humidity on changes in the processes of embryonic development of mulberry silkworm. The experiments substantiated a sharp drop in the percentage of caterpillar revival due to a decrease in the water content of eggs (Bessonova, 1977; Umarov et al., 2023) carried out selection work on reproductive traits in new silkworm lines resistant to hot climates (Bogoslavsky, 2009).

In our opinion, the solution to this problem should be studied starting from the embryonic stage of the development of breeding material, that is, by selecting caterpillars at the last stage of embryonic development. Therefore, it is necessary to establish a relationship between viability at the embryonic stage and in the postembryonic period. In this case, the main goal was to select the most viable genotypes of the breeding material at the embryonic stage. In this direction, V.A.Strunnikov (Strunnikov, 1959) conducted the first studies in the 90s of the twentieth century, and in these experiments, it was found that in families of pure breeds that were well revitalized against high temperatures, the viability of the caterpillar stage was also high.

MATERIALS AND METHODS

Experimental research was carried out in the laboratory of mulberry silkworm breeding at the Research Institute of Silkworm Breeding. The material used in this research was a population of new breeding lines, which differ from each other in cocoon weight and technological characteristics of the sheath. These lines were divided into three groups. The first group included small cocoon lines - Line 27 and Line 28, which have high technological properties of cocoon thread. The second group included medium cocoon lines - Line 30 (Parvoz 1 x Xorij) and Line 31 (Parvoz 2 x Xorij). The third group consisted of lines - Line 300 and Line 301, which produce large cocoons. This material was created in the laboratory "Breeding of Mulberry Silkworms" at the Scientific Research Institute of Sericulture.

The experiment began by analyzing all egg lying obtained during the spring brooding period of 2022. Eggs selected from each line of 100 eggs in February were placed in a thermostat under special laboratory conditions, the temperature was maintained at 34ºC, and only for 2 hours, each morning, the eggs were removed to room temperature during incubation. At the end of incubation, embryos that had reached the blastogenesis stage, the period of embryonic development in the egg, were counted. The next stage of experiments was continued in spring, in March-April. Spring incubation was carried out under moderately optimal hygrothermal conditions (24-25ºC and 75-80%). At the end of each incubation process, the percentage of egg revitalization was determined both in spring and winter.

RESULTS AND DISCUSSION

The mulberry silkworm is such a biological object that some complexity of selection on the viability of caterpillars characterizes it. If breeding families from the same breed are kept under the same optimal conditions, the viability of the breeding material will not differ much from each other. However, viability in lying resulting from the fusion of different parental gametes may also be manifested to different degrees. If a method of selecting strong genotypes (families) from multiple breeding families without caterpillar feeding is developed for breeding and pedigree work, selection for caterpillar viability in the breeding population can be much more effective and low-cost. In addition, by applying this method, it is possible to accelerate the process of selective breeding and achieve the goal. For this selection, various influences must be exerted on the egg later in the wintering of the eggs, when the eggs can already be revitalized. For example, to create extreme factors, eggs were kept in water for some time, exposed to hot water, and exposed to hydrochloric acid.

Table 1. Egg revitalization rates under critical and normal temperature conditions

Selection lines	The number of families analyzed		Percentage of revivability at critical temperature, (gradations)	Distribution of families by gradations	
				Quantity	Percent
Line 28	44	I gr	10.0-21.0	27	61.4
		II gr	22.0-34.0	15	34.1
		III gr	35.0-46.0	2	4.5
Line 27	62	I gr	21.0-30.0	23	37.1
		II gr	31.0-50.0	32	51.6
		III gr	51.0-61.0	7	11.3
Line 30	9	I gr	18.0-31.0	2	22.2
		II gr	32.0-45.0	3	33.3
		III gr	46.0-59.0	4	44.4
Line 31	14	I gr	33.0-49.0	4	28.6
		II gr	50.0-66.0	6	42.9
		III gr	67.0-84.0	4	28.6
Line 300	82	I gr	26.0-42.0	20	24.4
		II gr	43.0-61.0	44	53.2
		III gr	62.0-78.0	18	22.0
Line 301	83	I gr	23.0-39.0	29	34.9
		II gr	40.0-56.0	44	53.0
		III gr	57.0-72.0	10	12.0

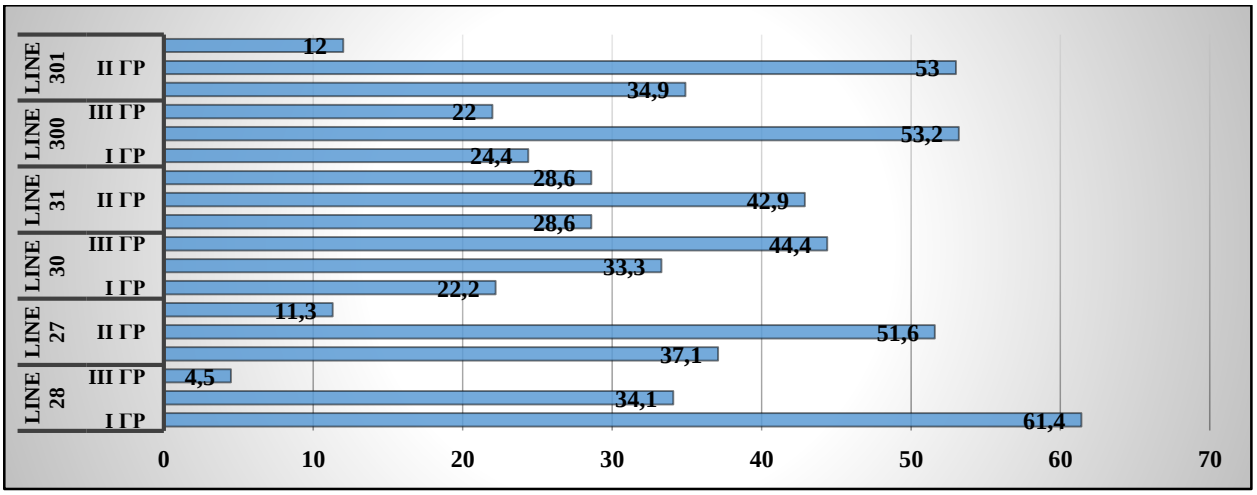

Fig. 1. Egg revitalization rates at critical and normal temperature conditions (Table 1).

The main objective was to identify strong genotypes that retain their embryonic viability under such a variety of severe exposures. Several experiments have found that incubation at high critical temperatures can allow the selection of resistant families among breeding silkworm breeds and lines. With this in mind, we conducted experiments aimed at identifying the strongest, most stable families. Families of breeds with different biological and technological properties under conditions of high-temperature exposure (+34ºC) maximally preserve their potential during embryonic development. The comparative performance of families of lines – Line 27, Line 28, Line 30, Line 31, Line 300, and Line 301 incubated at high critical temperatures is summarized in Table 1 and Fig. 1.

From the data in Table 1, it can be seen that some families of Line 31, Line 300, and Line 301 lines were revitalized from 72.0% to 84.0%. Within each breeding line, families' revivability at high temperatures was divided into three gradations. At the same time, the gradations for each line were made individually. For example, if we compare the relatively low-enlivened Line 28 with the well-enlivened Line 31, we can see that in Line 28, Gradation I indicators are distributed between 10.0-21.0%; Grade II, 22.0-34.0%; and Gradation III, 35.0-46.0%.

The most important thing for us in this experience was how the breeding families of each line would be graded. That is, how many families revitalize well or poorly at critical temperatures? The table clearly shows that only 4.5% of the 44 families in Line 28 were revitalized in the Gradation III section, and this figure was 44.4% in Line 30. The proportion of well-animated families in the III gradation of other lines was 11.3%; 12.0%; 22.0%; and 28.6%, respectively.

CONCLUSIONS

For the first time in mulberry silkworm breeding, the effect of stress temperatures on the viability of embryos in breeding populations was detected. The fact that breeding lines respond differently to critical temperature levels indicates that mulberry silkworm genotypes are resistant to an unfavorable factor, such as varying degrees. Our initial experimental results confirm that Line 30 and Line 300 are resistant to critical doses of temperature during embryonic development.

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Diagnosis of pelvic floor and pelvic organ prolapse by ultrasound examination

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Unified and systematized ultrasound hidden (preclinical) and clear signs of prolapse of the pelvic floor and all parts (anterior, middle and posterior) of the small pelvis. On the basis of displacement and deformation of organs, thickening of their walls, anatomical changes in the ligamentous-muscular structures, geometric and topometric deviations, isolated and concomitant prolapse of the pelvic organs was established. A three-stage ultrasound study was proposed, which contributed to the identification of the severity of prolapse and the establishment of its stage and the choice of the most rational therapeutic (surgical) tactics and its volume.

Keywords: *Pelvic prolapsus, prolapse of the rectum, rectocele, urethrocele, prolapse of the uterus, ultrasound semiotics*

INTRODUCTION

In clinical practice, sagging (falling) of the pelvis and small pelvic organs are isolated and in common cases - rectocele, rectum (rectum) and / or uterine (uterine rupture, uterine cervix, uterine cervix, uterine arch) sagging, bowel. They cover the most important sections of surgical coloproctological and urogynecology, with varicose veins of the upper extremities (enterocele), urethrocele, cystocele, urethral cystocele and others (Olsen et al., 1997). The prevalence of small pelvic organ prolapses (PVL) in adults ranges from 3-8% to 56% (Susan and Barbara B, 2005), and is more common in women over 80 years of age (Maher et al., 2008). In women around the age of 40, this figure is 30% (De Lancey et al., 2008; McLennan et al., 2006; Niggard et al., 2008). The peak of the disease corresponds to the age of 60-69 (McLennan et al., 2006). The frequency of pathology in the postmenopausal age varies between 60-80% (McLennan et al., 2006; Niggard et al., 2008). According to most authors, the actual figures are very different from the literature, as asymptomatic patients and some women do not go to health facilities due to national-ethnic mentality and customs (Horst et al., 2017; Лологаева et al., 2019.) Pelvic prolapse is almost always manifested by dysfunction of the pelvic organs at one level or another: failure to hold defecation (feces and gas), sexual sphere (painful sexual intercourse), urinary excretion due to the lower urinary tract (lack of urinary retention) disorders, etc. (Abhyankar et al., 2019). The Department of Gynecology at the University of Toronto (Canada) has called the ICU a "hidden epidemic" of the century, based on a report on demographics. M.D.Moen found that anatomical abnormalities of the pelvic floor needed operative correction in 11% of the female population. Thirty percent of these women undergo repeated surgeries due to the recurrence of the disease (Marinkovic and Stanton, 2004).

Despite significant advances in diagnostic technology and tools, serious challenges remain in the recognition of pathology. According to M.Barber and M.Maher (2013), only 3-6% of patients in the early stages see a doctor with clinical symptoms, but up to 50% of patients show diagnostically significant symptoms on vaginal

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examination, including ultrasound (Kayembe et al., 2024).

The purpose of the study. Study and diagnostic evaluation of ultra-sonographic semiotics of isolated and joint oscillations of the pelvic floor and small pelvic organs.

MATERIALS AND METHODS

The study included 85 patients aged 16-87 years (average age in women - 48.1 ± 1.3 years, men – 51.32 ± 1.8 years) with rectal prolapse of varying severity at the AMU TCC in 2014-2021 - 54 (63.5%)). 41 women (75.9%) reported a history of severe traumatic birth. Ultrasound examination was performed in the morning on an empty stomach, without special training, with a device "Alake" SSD-630 (Japan), superficial, rectal and / or vaginal transmission (with sensors). We support the purposeful conduct of the examination based on the patient's complaints (fecal incontinence, diarrhea, constipation; painful sexual intercourse, foreign body sensation; urinary incontinence, painful, intermittent and intermittent urination).

The following exosonographic signs of pelvic floor and pelvic organ sagging were detected during USG. Distal displacement of the rectum, uterus, cervix, uterus, bladder relative to the groin, deformation of these organs or some of them, wall thickening, flattening of the rectum, dilation of the anus, anal canal, uterine dilatation, bladder uterus Displacement of the rectum towards the uterine tract or backward (rectocele)

Methods of perineal (middle) ultrasound examination. The examination is not related to the menstrual cycle, and we recommend that it be performed at any time, in the morning and on an empty stomach (which worsens the appearance of bloating after a meal). The bladder should be 100-150 ml full. An overcrowded bladder can distort the results by squeezing the pelvic organs, and an overcrowded bladder can cause difficulties in visualizing neighboring organs and elements. The patient is stretched on his back on the gynecological table, raises his legs by bending at the knees and squeezes the upper extremities to the abdomen.

Images (topography) and tonometry of the rectum on intermediate USG. The anal canal, the attitude of the rectum to the uterine tract, the deformations along the course, the bulge towards the uterus (rectocele) are studied. The anorectal angle is the angle formed by the intersection of the longitudinal axis of the anal canal and the line passing along the posterior wall of the distal branch of the rectum. USG allows you to determine the dimensions of the angle. The relationship of the anorectal region to the groin line (imaginary line connecting the symphysis with the groin) is studied. Swelling of the anterior or posterior walls of the rectum anteriorly (towards the bladder in men, towards the uterus in women) and posteriorly with respect to normal gait is determined during rest and relaxation (Valsalva test). Intermediate USG allows for assessment of the condition of the mesorectum, parametrium, Para vesical and Para urethral area, rectovaginal septum (rectovaginal fistula), retro rectal area (Perirectal fistulas), pathological, including volume derivatives. USG examination of the rectum reveals deformities, thickening, external compression, swelling, sagging, and volume derivatives, sometimes a fistula, or even an internal hole in the anterior and posterior walls. Examination of the distal retroperitoneal part of the organ reveals fascial defects at the level of the dentate gyrus, dilatation of the rectovaginal septum (diastasis of the anterior and posterior groups), and m. Different degrees of swelling due to layering of the mus. levator any (diastases of the clusters) - rectocele is visible. However, data on perineal USG are limited compared to end rectal and transvaginal USG examinations.

Ultrasound visualization, topography of the uterus, cervix, uterine tract and arch. **Ultrasound visualization,** appearance of the uterus, cervix, uterine tract and arch, displacements, deformations, compression of external organs, their symphysis, seminal vesicles, urethra and rectum (anal canal) are studied at rest and during exertion (Valsalva test) (Fig. 1).

Ultrasound imaging, topography and tonometry of the bladder and lower urinary tract. The smoothness of the bladder walls, roughness, thickening, derivatives in the bladder, compression by the surrounding organ, etc. Ultrasound signs appear. Length of the urethra (distance from the neck of the bladder to the symphysis), then the angle of inclination of the urethra (α), posterior bladder - the urethra (β),

pubic-urethral (angle of intersection of the axis passing through the symphysis) (γ), from the symphysis the angle δ formed by the intersection of the horizontal axis with the line passing through the support area of the bladder is measured (Fig. 2).

Fig. 1. Ultrasound image of a prolapsed uterus.

Fig. 2. Ultrasound image of a prolapsed Vesical seller.

Endorectal and transvaginal Ultrasound examinations were performed by us on a multi-stage basis. On endorectal Ultrasound examination:

I stage:

- Determination of the completeness and weakness of the anal sphincter muscles (expansion, contraction, scarring, etc.);
- pelvic diaphragm muscles, m. study of the condition of the levator ani (layering, diastases, scars and scar deformities);
- completeness, weakness of the rectovaginal wall (layering, diastasis, fistula and perforations);
- the height of the gap (distance from the anal opening to the bottom of the rectum-bladder (in men), rectum-uterus and uterus-bladder (in women)).

On transvaginal USG:

- completeness, weakness of the rectovaginal wall (layering, diastasis, fistula and perforations);
- condition of the pelvic diaphragm.

Stage II USG: - 3D-reconstruction of anal sphincter muscles and internal sphincter muscle of urethra and assessment of their condition.

Stage III USG: The condition of the rectum, the anterior and posterior walls of the uterus, the length, width and wall thickness of the urethra, the condition of the cervix, the bottom of the bladder are studied. At this stage, swelling of the posterior wall of the bladder (cystocele), anterior (anterior rectocele) and posterior walls (posterior rectocele) of the rectum, as well as the length and degree of flexion of the urethra, the symphysis of the uterine cervix is determined at rest.

Localization and demonstration of pelvic floor and limb sagging in the USG. The following localizations of anatomical USG-changes of the urethrocele in the anterior compartment have been identified: in the anterior, middle, and posterior sections of the pelvis, systole and urethrocystosele (the anterior wall of the uterus may also be involved in the process); in the middle section, there is a fall of the arch of the uterus (occurs after amputation or extrapolation of the uterus), joint sagging of the uterus and neck (apical prolapse), enterocele (small bowel loops with competition) and in the posterior rectocele, rectum fall, enterocele (Fig. 3).

According to the POP-Q classification (1996), the stages of prolapse can be determined on the basis of USG data:

• Stage I – the bottom of the swinging part of the limb is located 1 cm above the hymen ring;

• Stage II – the bottom of the swinging part of the limb is located 1 cm above the end of the uterine corridor;

• Stage III – 2/3 of the uterine tract protrudes from the genital cleft;

• Stage IV – the uterus goes completely out (Persu et al., 2011).

Statistical analysis. The incidence of events (ultrasound signs) in cases of pelvic floor and pelvic floor prolapse was calculated by performing a multiple chance analysis of the images (ultrasound clinical signs):

$$\mathbf{C}=\frac{p}{1-p}$$

Here: C – chance of encountering ultrasound signs; p – the probability of occurrence of ultrasound signs in the studied collection.

For the comparison and statistical evaluation of the obtained indicators, the odds ratio (OR) and the 95% confidence interval (CI) of this indicator were calculated in the comparison groups.

$$\mathbf{OR}=\frac{ad}{bc}$$

$$\mathbf{CI}=e^{lnOR\pm 1{,}96\sqrt{\frac{1}{a}+\frac{1}{b}+\frac{1}{c}+\frac{1}{d}}}$$

Here: a, b, c and d are numbers in a 4-cell table; OR − odds ratio; CI − 95% confidence interval; 1.96 − the crisis price corresponding to the p=0.05 level of the standard normal distribution.

Fig. 3. USG image: Enterocele.

If the OR indicator is less than 1, it means a decrease in chance, if it is greater than 1, it means an increase, if it is equal to 1, there is no difference. The inclusion of OR=1 or 1 in the CI means that the difference between the indicators in the groups is not statistically correct. If OR>1 and 1 is not included in the EI, the decision is made that the studied event met with a statistically correct high chance. On the contrary, if OR<1 and 1 is not included in the CI, the chance of encountering the ultrasound sign is estimated as statistically incorrect.

Normally, the anorectal angle ranges from 85-95^0, and during exacerbation, it ranges from 100-110º. The angle is blind in rectal prolapses. When the angle is in the range of 110-115º, when it is reconciled with clinical data and other instrumental examinations (X-ray defecography), we consider it as grade I, 115-125^0 - II, and more than 125º as grade III sagging (normal indicators are taken on the basis of literature data) (Persu et al., 2011; Walters and Daneshgari, 2004). In silence during perineal USG, the anorectal region should be located up to 3 cm below the imaginary line of the inguinal crease, and more than 3 cm during excitation. If this indicator is ≤3 cm in silence and ≤4-5 cm during exacerbation, it is considered prolapse of the rectum. The degree of sagging depends on the size of the distance: up to 3.5 cm - I, 3.5-4.5 cm - II, 5-6 cm and more - III degree (OR>1 and 1 are not included in CI, i.e. the results are statistically correct).

Normally, during perineal USG, the neck of the uterus is not visible in standard views (projections). The fact that the neck appears above the symphysis at rest and does not descend below the symphysis in the Valsalva test is consistent with grade I drooping of this limb; at the time of exacerbation, the length of the uterus descends up to 2 cm below the symphysis II; when it is visible at the lower edge of the symphysis at rest and when it is strong it goes down to the external opening of the uterus, which corresponds to grade III (OR>1 and 1 is not included in the CI, it means, the results are statistically correct).

The condition of the ligamentous apparatus of the neck of the bladder allows studying the condition of both it and the muscles of the pelvic diaphragm. At rest, the shortening of the distance from the neck of the sac to the symphysis is lig. the vertical part of the pubocervical, and the greater shortening of the m. puborectalis, m. decrease in the tone of the muscles of the pubococcygeus and the anterior wall of the uterus, it means, symptoms of cystocele and sagging of the cervix (OR=1 and 1 is included in the CI, that is, the result is not statistically correct).

Perineal, endorectal, and transvaginal USG provides diagnostic and differential diagnosis of pelvic floor condition and limb sags, pathologies; determination of pelvic limb sagging and degrees of severity; selection of surgical tactics, volume and method; in the diagnosis of organic causes of urinary incontinence in women; assessment of end prosthesis condition, migration, shrinkage; control of the condition of the pelvic floor in the period after the surgical operation; diagnosis of concurrent and postoperative diseases and complications (rectovaginal and anorectal fistulas, abscesses, volume processes, recurrences), the ability to provide an assessment of the results of surgical treatment (Drutz and Alarab, 2006).

The sonographic sign of diastases of the levator muscles is the appearance of swelling, m. is a clear and precise visualization of the transverse perinea superficial is muscle (OR>1 and 1 is not included in CI, it means, the result is statistically correct).

The thickness of the fibers of the diaphragm muscle in women without sagging of the pelvic floor and limbs is not less than 15 mm, and in men it is not less than 12 mm (OR>1 and 1 is not included in CI), it means, the result is statistically correct. A low index is one of the indirect signs of muscle failure.

DISCUSSION

Perineal, endorectal and transvaginal US provides diagnostic and differential diagnosis of the state of the pelvic floor and sagging of its limbs, pathologies; determination of pelvic limb sagging and degrees of severity; selection of surgical tactics, volume and method; in the diagnosis of organic causes of urinary incontinence in women; assessment of endoprosthesis (mesh) condition, migration, shrinkage; control of the condition of the pelvic floor in the period after the surgical operation;

besides, it has the ability to provide diagnosis of post-operative diseases, complications (rectovaginal and anorectal fistulas, abscess, volume processes, recurrence events), evaluation of the results of surgical treatment.

CONCLUSIONS

USG semiotics of pelvic floor insufficiency and pelvic organ sagging: anal and urethral sphincter, thinning of the diaphragmatic muscle groups, scarring, deformation of the tendon of the diaphragm, m. Diastases of the levator ani, layering, swelling of the contours to the neighboring organs (bladder, uterus) (rectocele), displacement of the pelvic organs to the symphysis, the imaginary lines passing through the symphysis, changes in distance and angle, etc. These symptoms can be considered the first preclinical, latent and early signs of pelvic floor musculoskeletal insufficiency. USG of the pelvis and limbs reveals the preclinical signs of prolapse, opens a wide range of opportunities for preventive and therapeutic (intermediate gymnastics, biological feedback therapy, etc.) and dynamic follow-up, planning and selection of surgical treatment (tactics, scope and method).

1. When reconciling with clinical data and other instrumental examinations (RH-ji defecography), if the angle is within the limits of 110-1150, we consider it as grade I, 115-1250 - II, and more than 1250 as grade III sagging. Normal indicators are presented in the literature [Persu et al., 2011; Susan et al., 2005]. Normally, the anorectal angle ranges from 85-950, and during exacerbation, it ranges from 100-1100. The angle is blind in rectal prolapses.
2. During perineal USM at rest, the anorectal region should be located up to 3 cm below the imaginary line of the inguinal fold, and more than 3 cm during exacerbation. If this indicator is ≤3 cm at rest and ≤4-5 cm during exacerbation, it is considered prolapse of the rectum. The degree of sagging depends on the size of the distance: up to 3.5 cm - I, 3.5-4.5 cm - II, 5-6 cm and more - III degree (SN>1 and 1 are not included in EI, i.e. the results are statistically honest).
3. The appearance of the cervix above the symphysis at rest and not descending below the symphysis during the Valsalva test corresponds to the I-degree prolapse of this member: at the moment of exacerbation, the cervix descends up to 2 cm below the symphysis II; appearing at the lower edge of the symphysis at rest and descending to the external opening of the uterus during exacerbation corresponds to grade III (SN>1 and 1 are not included in the EI, that is, the results are statistically honest). Normally, during perineal USM, the neck of the uterus is not visible in standard views (projections).
4. The sonographic sign of diastasis of the levator muscles is the appearance of swelling, m. is a clear and accurate visualization of the transverse perinea superficial muscle (SN>1 and 1 is not included in the EI, that is, the result is statistically honest).
5. A decrease in the thickness of the fibers of the pelvic diaphragm muscle is one of the main sonographic signs of the failure of that muscle. The thickness of the fibers of the pelvic diaphragm muscle is 15 and 12 mm higher in healthy women without sagging of the pelvic floor and limbs (SN>1 and 1 are not included in the EI, that is, the result is statistically honest.
6. The condition of the ligamentous apparatus of the neck of the bladder allows studying the condition of both it and the muscles of the pelvic diaphragm. At rest, the shortening of the distance from the neck of the sac to the symphysis is lig. the vertical part of the pubocervicale, and the greater shortening of the m. puborectalis, m. pubococcygeus and anterior wall of the uterus are signs of a decrease in tone, that is, a cystocele and a sagging cervix (SN=1 and 1 are included in the EI, that is, the result is not statistically honest).

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Prevalence of rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene in patients with acute brucellosis depending on the damage to the hepatobiliary system

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Human genetic factors play an important role in the present stage of studying new aspects of the pathogenetic mechanisms of development, course, and outcomes of infectious diseases. Some studies established that the Asp299Gly polymorphism of the TLR-4 gene, which is currently associated with a variety of diseases, is of clinical importance. The article presents the results of an examination of 120 patients with acute brucellosis. The diagnosis of brucellosis was made based on complaints, anamnesis, epidemiologic and clinical data, and the results of specific examinations. The Asp299Gly polymorphism of the TLR-4 gene was determined in all patients. A detailed characterization of patients with brucellosis has been presented. Young, working-age men prevailed among the examined persons. Carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene were found to have an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4.25-38. 29]), and in the case of carrying the A allele, on the contrary, they had a decreased risk of acute brucellosis with liver damage (OR=0.0895% CI [0.03-0.24]) model significant at $\chi 2$ =27.87P < 0.0001). Carriage of homozygous genotype A/A had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07]).

Keywords: *Acute brucellosis, TLR-4, polymorphism, gene, liver*

INTRODUCTION

Brucellosis is considered one of the most common bacterial zoonoses worldwide and remains a serious problem not only for public health systems in many countries of the world but also for veterinary medicine in particular (Di Bonaventura et al., 2021; Nicoletti and Paul 2010; Laine, 2023; Yu et al., 2024).

The problem of brucellosis is the lack of adequate statistical data on the true incidence of the disease in the human population, as a consequence of the lack of vigilance against this disease, as well as in the delayed diagnosis, untimely and inadequately prescribed specific treatment, which in turn leads to an increase in the number of chronic cases of brucellosis after completion of etiotropic therapy (Franco et al., 2007; Saha, et al., 2013).

Brucellosis is a disease characterized by a wide variety of clinical manifestations with possible involvement of almost any organ of the human body and a wide range of so-called clinical "masks", which leads to the untimely and delayed establishment of the correct diagnosis (Adone and Pasquali 2013; Al Dahouk et al., 2013).

Moreover, Brucella species can persist for extended periods within phagocytes, evade detection by the immune system, and establish chronic infections (Dominguez-Flores et al., 2023; De Jong et al., 2012). The enduring presence of Brucella leads to the suppression of mononuclear apoptotic cells, hindrance of dendritic cell maturation, decreased presentation

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of antigens, and deactivation of T-cells (Ganji et al., 2017). The host's immune system encounters delays in identifying Brucella due to alterations or complete suppression of pathogen-associated molecular patterns (Mazlan et al., 2021). Numerous research studies have been carried out to explore the resistance of B. melitensis to antibiotics like rifampicin and trimethoprim / sulfamethoxazole (Barbosa et al., 2015; Wareth et al., 2022). As a result, the identification of new targets against B. melitensis remains highly significant (Pradeepkiran et al., 2021).

As a result of ingestion and development of brucellosis infection in the human body, immunologic restructuring of the whole organism occurs. At the present stage, there are still many gaps regarding the complete understanding of all pathogenetic links in the formation of postinfection immune responses. First of all, the issues of intercellular relationships in the process of immune response formation remain open (Ahmed, et al., 2016).

Unlike other pathogens, the brucellosis pathogen is not characterized by classical pathogenic factors such as exotoxins, exoproteases, cytolysins, and other exoenzymes that cause direct damage to human cells (Moreno, 2002). Hence, it is logical that in brucellosis, tissue damage may result from indirect mechanisms, probably due to activation of host immune responses following recognition of the brucellosis pathogen by immune system receptors such as TLRs. It was found that TLR-2, TLR-4, and TLR-9 may participate in the recognition of brucellosis by phagocytes (Oliveira 2008; Dominguez-Flores, 2023; Yu et al., 2024).

One of the main reasons influencing any changes in TLR immune response in brucellosis is considered to be genetic polymorphism of these receptor genes (Molteni et al., 2016; Netea et al., 2012).

The role of TLR-4 polymorphism and its association with brucellosis is an ambiguous and poorly studied problem. Currently, there is single data on this issue, so in one study the polymorphism (Asp299Gly) of the TLR-4 gene in brucellosis patients was analyzed. It was concluded that the G allele was more prevalent in patients with brucellosis compared to healthy individuals (33.6% vs. 20.7%, p=0.000003). Thus, this study first established an association between genetic polymorphism of the TLR-4 gene and susceptibility to brucellosis (Rezazadeh et al., 2006).

To determine the frequency of the TLR-4 gene polymorphism rs4986790 (Asp299Gly) in patients with acute brucellosis in the Republic of Azerbaijan, taking into account liver damage.

MATERIALS AND METHODS

We examined 120 patients with clinical brucellosis who sought medical care at Baku Clinic and Baku Central Clinical Hospital. All patients gave written permission to be included in the study.

The diagnosis of acute brucellosis was established based on clinical data, anamnesis, including epidemiologic, and objective examination data, and results of laboratory diagnostics, including specific diagnostics.

The diagnosis of acute brucellosis was established based on clinical and epidemiological data, anamnesis, and data from an objective examination, results of laboratory diagnostics, including specific ones.

Specific research was carried out by ELISA on Awareness and Stat Fax 3200 devices using NovaLisa Brucella IgG, IgM test systems (Germany) with the detection of IgM and IgG.

The criteria for inclusion in the study were a diagnosis of acute brucellosis, and the duration of clinical symptoms was taken into account, namely up to 3 months from the onset of the first complaints.

The main group consisted of 120 individuals who fully met the inclusion criteria for the study. The control group consisted of 30 practically healthy individuals who underwent a scheduled annual examination. The groups were representative by age and gender. Patients of both groups are ethnic Azerbaijanis who permanently reside in the Republic of Azerbaijan. The average age of patients in the main group was 35.9±2.8 years. Among those examined, males predominated – 75.0%.

All 120 patients with acute brucellosis were divided into three subgroups according to the disease severity. The following symptoms served as severity criteria: fever, sweating, chills, headache, insomnia, decreased blood pressure,

tachycardia, hepatosplenomegaly, myocarditis, pericarditis, endocarditis, changes in the general blood count, levels of pro-inflammatory and anti-inflammatory cytokines. Thus, a mild degree was diagnosed in 74 (61.7%) patients, a moderate degree - in 35 (29.1%) patients and only 11 (9.2%) patients had a severe condition.

To assess the liver condition, all patients underwent biochemical studies with the mandatory determination of total bilirubin, ALT, AST, ALP, GGT, and LDH levels, and also ultrasound examination of abdominal cavity organs was performed on a Voluson E8 General Electric apparatus using 4D convex 4-8 MHz RAB 4-8D volumetric multifrequency transducer.

All patients were screened for markers of viral hepatitis A, B, C, D, and E. Hepatitis of non-viral etiology, namely autoimmune, toxic, and alcoholic, was also excluded.

The TLR-4 gene polymorphism (Asp299Gly) was tested in all patients. Genomic DNA was extracted from peripheral blood mononuclear cells using a DNA extraction kit according to the manufacturer's instructions. Amplifications followed by electrophoretic separation of amplification products of the corresponding gene were used to identify polymorphic alleles. Amplification was performed on an iCycler IQ5 amplifier (BioRad, USA).

The amplification mode was as follows: 93°C, 1 min; 35 cycles: 93°C, 10 sec; 64°C, 10 sec, 72°C, 20 sec; 72°C, 1 min.

The reliability of differences in the distribution of genotypes at polymorphic loci between the groups was tested by Hardy-Weinberg equilibrium (http://gen-exp.ru/calculator_or.php).

Statistical processing of the study results was performed using the programs "SPSS 20.0" and "STATISTICA 6.0". A Comparison of genotype and allele frequencies was performed by analyzing conjugation tables using Fisher's exact test and the $\chi 2$ criterion depending on the analysis assumptions. The risk of pathology development was assessed by calculating the odds ratio (OR) with a 95% confidence interval (CI) by simple logistic regression.

RESULTS AND DISCUSSION

Among patients with acute brucellosis, the normal serum ALT level was detected much more frequently ($p<0.05$), namely in 64.17±4.38% (77 patients), while elevated in 35.83±4.37% (43 patients). The AST level in serum was found to be above normal in 40.00±4.47% (48 patients), while the normal level was found in 60.00±4.48% (72 patients).

We believe the higher AST level in these patients is attributed to the high frequency of cardiovascular lesions, namely in 77.5% of individuals (Fig.1).

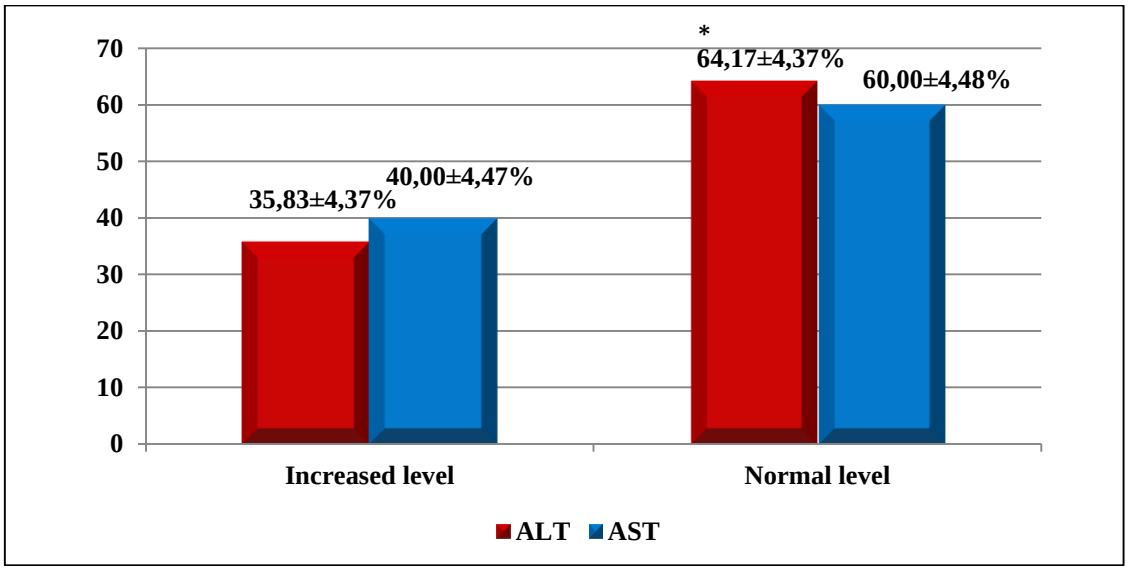

Fig. 1. Distribution of patients with acute brucellosis depending on serum ALT and AST levels.

Table 1. Analysis of the odds ratio for the increase in the level of cytolysis indicators in the serum of patients with acute brucellosis, depending on gender

Patients with acute brucellosis (n=120)	Males (n=90)		Females (n=30)		Differences between groups, p	OR (95% CI)
	Abs.	%	Abs.	%		
Patients with elevated ALT levels (n=43)	38	42.22±5.21	5	16.67±6.80*	0.015	3.65 [1.28-10.41]
Patients with normal ALT levels (n=77)	52	57.78±5.20	25	83.33±6.81		
Patients with elevated AST levels (n=48)	46	51.11±5.27	2	6.67±4.56*	0.0004	14.64 [3.29-65.13]
Patients with normal AST levels (n=72)	44	48.89±5.27	28	93.33±4.55*		

Note. * - $p < 0.05$ - statistically significant difference between male and female patients with acute brucellosis.

Table 2. The frequency of detection of the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene in patients with acute brucellosis depends on the severity degree

Genotypes TLR-4 (Asp299Gly)	Patients with acute brucellosis (n=120)					
	Mild degree (n=74)		Medium degree (n=35)		Severe degree (n=11)	
	Abs.	%	Abs.	%	Abs.	%
Genotype A / A	33	44.59±5.77	2	5.71±3.92 *	0	0
Genotype A / G	40	54.06±5.79	33	94.29±3.92*	9	81.82±11.62
Genotype G / G	1	1.35±1.30	0	0	2	18.18±11.62

Note. * $p < 0.05$ - between patients with acute brucellosis with different degrees of severity.

Gender-dependent peculiarities in patients with acute brucellosis with normal and elevated levels of transferases in serum were established. Thus, males had elevated levels of transferases 2.5 times more frequently compared to females. The same tendency was observed concerning the gender distribution of the AST level. Thus, the AST level elevation was registered only in 2 female patients, which amounted to 6.67±4.56%, compared to 51.11±5.27% male patients.

Male gender among patients with acute brucellosis was found to be associated with more frequent detection of the elevated serum ALT level (OR=3.65; 95% CI [1.28-10.41]; p=0.015). The same association was found for elevated serum AST (OR=14.64; 95% CI [3.29-65.13]; p=0.0004) (Table 1).

The A/G genotype of the TLR-4 gene was detected almost 1.74 times more frequently in patients with moderate acute brucellosis than in patients with mild acute brucellosis.

Acute brucellosis was significantly more frequent (7.9 times) in carriers of homozygous genotype A/A, compared to patients with acute brucellosis of medium severity ($p<0.05$). No significant differences were found among carriers of the G/G genotype with different degrees of severity (Table 2).

The A/A genotype of the TLR-4 gene was detected in 44.59±5.77% of patients with a mild course of acute brucellosis, whereas among patients with moderate severity, this genotype was detected only in 5.71±3.92%.

No patient with a severe course of acute brucellosis was found among carriers of genotype A/A of the TLR-4 gene. In contrast, acute brucellosis was usually severe or moderately severe in carriers of the A/G genotype of the TLR-4 gene.

When analyzing the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene, a reliable difference was found only in carriers of homozygous genotype A/A between acute brucellosis patients and practically healthy individuals ($p <0.05$). Thus, genotype A/A of the TLR-4 gene was detected 3.4 times more frequently in patients with acute brucellosis without signs of liver damage than in patients with liver damage.

The A/A genotype of the TLR-4 gene was detected in 11.62±4.88% of individuals with acute brucellosis with liver damage, while in the case of acute brucellosis without signs of liver damage, it was observed in 38.96±5.56% of the patients. Among carriers of other genotypes, no significant difference was found in the groups of patients with and without liver damage.

Table 3. Frequency of detection of the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene among patients with acute brucellosis, depending on liver damage and in practically healthy individuals

Genotypes and alleles TLR-4 (Asp299Gly)	Acute brucellosis patients with liver damage (n=43)		Acute brucellosis patients without liver damage (n=77)		Healthy individuals (n=30)	
	Abs.	%	Abs.	%	Abs.	%
Genotype A / A	5	11.62±4.88	30	38.96±5.56 *	27	90.00±5.47*
Genotype A / G	35	81.40±5.93	47	61.04±5.56	2	6.67±2.27*
Genotype G / G	3	6.98±3.88	0	0	1	3.33±1.64
Allele A	45	52.33±5.38	107	69.48±3.71	56	93.33±3.22
Allele G	41	47.67±5.38	47	30.52±3.71	4	6.67±3.22 *

Note. * P < 0.05 - between patients with acute brucellosis with and without liver damage and healthy individuals.

Table 4. Association of alleles and genotypes for the rs4986790 (Asp299Gly) polymorphism of the TLR-4 gene and susceptibility to acute brucellosis with liver damage

Alleles and genotypes	Acute brucellosis patients with liver damage (n=43)	Healthy individuals (n=30)	χ^2	p	OR	
					Values	95% CI
Allele A	0.523	0.933	27.87	<0.0001	0.08	0.03 - 0.24
Allele G	0.477	0.067			12.76	4.25 - 38.29
Genotype A/A	0.116	0.900	44.65	0.001	0.02	0.01 - 0.07
Genotype A/G	0.814	0.067			61.25	12.03 - 311.76
Genotype G/G	0.070	0.033			2.18	0.22 - 21.98

Table 5. Dominant inheritance model of variant allele at polymorphism rs4986790 (Asp299Gly) in acute brucellosis patients with liver damage

Genotypes	Acute brucellosis patients with liver damage (n=43)	Healthy individuals (n=30)	χ^2	p	OR	
					Values	95% CI
Genotype A/A	0.116	0.900	44.09	<0.0001	0.02	0.01-0.07
Genotypes A/G +G/G	0.884	0.100			68.40	15.05-310.87

The G allele was significantly more frequent (7.2 times) in acute brucellosis patients with liver damage than in healthy individuals and 4.6 times more frequent in acute brucellosis patients without liver damage ($p < 0.05$) (Table 3). Thus, in acute brucellosis patients with liver damage, the frequency of allele G was 47.67±5.38%, whereas in healthy individuals, this allele was detected only in 6.67±3.22% of cases.

The next stage was to determine the risks of acute brucellosis with liver damage, taking into account the carriage of polymorphic variants of the TLR-4 gene. When studying the allele frequency distribution in patients with acute brucellosis, it was found that carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene had an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4. 25-38.29]), while in the case of carrying the A allele, on the contrary, the risk of acute brucellosis with liver damage was reduced (OR=0.0895% CI [0.03-0.24]), the model is significant at $\chi2 =27.87P < 0.0001$) (Table 4).

The association of genotypes for polymorphism rs4986790 (Asp299Gly) of the TLR-4 gene and susceptibility to acute brucellosis with liver damage was also established. We found, a significantly increased risk of acute brucellosis with liver damage among carriers of the A/G genotype of the TLR-4 gene ($\chi2 =44.65$; $p=0.001$; OR =61.25; 95% CI [12.03-311.76]), whereas carriage of the homozygous A/A genotype, on the contrary, had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07]).

When analyzing the total frequency of A/G +G/G genotypes in acute brucellosis patients with liver damage and practically healthy individuals, we used the dominant model of inheritance of acute brucellosis (Table 5).

The combination of A/G +G/G genotypes in acute brucellosis patients with liver damage was detected 8.8 times more frequently than in practically healthy individuals (OR =68.40% CI [15.05-310.87]; $\chi 2$=44.09, p<0.0001).

CONCLUSIONS

1. In patients with acute brucellosis, the male gender was associated with a higher incidence of elevated serum ALT levels (OR=3.65; 95% CI [1.28-10.41]; p=0.015).

2. In carriers of homozygous genotype A/A, acute brucellosis was much more frequently (7.9 times) milder than in patients in whom the course of brucellosis was considered to be of medium severity (p<0.05).

3. Carriers of the G allele of the polymorphic (Asp299Gly) TLR-4 gene had an increased risk of acute brucellosis with liver damage (OR=12.76, 95% CI [4.25-38.29]), and conversely, carriers of the A allele had a decreased risk of acute brucellosis with liver damage (OR=0.0895%, CI [0.03-0.24]) model significant at $\chi 2$ =27.87, P < 0.0001).

4. Carriage of the homozygous genotype A/A had a protective effect against the development of acute brucellosis with signs of liver damage (OR =0.0295% CI [0.01-0.07])

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Clinical analysis of the effect of preeclampsia on the course of pregnancy and childbirth in multiparous women with iron deficiency anemia

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According to the World Health Organization, iron deficiency in the human organism is the most widespread anemia in the world. There is no country today that has studied and solved the problem of anemia completely. To analyze the impact of preeclampsia on pregnancy and childbirth in multiparous women with iron deficiency anemia. The study was conducted on 80 pregnant women aged 18-44 years. The objects of the study were multiparous women with iron deficiency anemia without preeclampsia 61 (76.3%) and with preeclampsia 19 (23.8%). Clinical-anamnestic information was obtained. A general analysis of blood and urine and a biochemical analysis of the pregnant women were performed. Methods of mathematical and statistical analysis- discriminant and variance analysis methods. Complicated obstetric anamnesis in pregnant women with iron deficiency anemia was observed in 73.7% of the women with preeclampsia and in 41.0% of the women without preeclampsia ($P_{\chi 2}$=0.013). Complicated gynecological anamnesis was in 3.3% of the women without preeclampsia and in 15.8% of the women with preeclampsia ($P_{\chi 2}$=0.049). IUGR was observed in 3.3% of the women without preeclampsia and 21.1% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.010). According to the comparative analysis, mild, moderate, and severe anemia occurred in 17 (27.9%), 43 (70.5%), and 1 (1.6) pregnant women without preeclampsia, and in 6 (31.6%), 11 (57.9%), and 2 (10.5%) pregnant women with preeclampsia, respectively ($P_{\chi 2}$=0.177). With the increasing severity of anemia, preeclampsia and cesarean delivery cases increased. The study of the effect of preeclampsia on the course of pregnancy and childbirth in pregnant multiparous women with iron deficiency anemia revealed that a complicated obstetric and gynecological history of pregnancy is predominant in this group of women. Surgical delivery is very common. It can be concluded that complicated obstetric and gynecological anamnesis is a premorbid background for the disease.

Keywords: *Multiparous women, preeclampsia, iron deficiency anemia*

INTRODUCTION

According to the WHO, iron deficiency is very common in the world population. Research is being conducted around the world to study this problem.

Iron deficiency anemia is a hematologic syndrome characterized by impaired hemoglobin synthesis as a result of iron deficiency, manifestations of sideropenia and anemia, as well as the development of trophic disorders in organs and tissues (Dobrokhotova et al., 2016).

Preeclampsia is a disease occurring during pregnancy with a lethal outcome in 5-8% of cases. Although effective diagnostic and treatment methods were improved to prevent the disease, the relationship between the capacity of pathological mechanisms and the variety of factors affecting pregnancy needs to be studied (Guillermina, 2017).

Preeclampsia is a multifactorial disorder characterized by hypertension and increased

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urinary protein excretion in pregnant women. It is the main complication that causes maternal and fetal deaths worldwide. Despite the controversial opinions on the pathogenesis of preeclampsia, the issue of predicting the initial symptoms of the disease has not yet been solved (Fatemeh al., 2018).

Preeclampsia is a disorder characterized by hypertension and proteinuria. According to the collected evidence, it is an endothelial disease. Angiogenic factors regulate the development of placental vessels. There is no evidence of the preeclampsia onset prediction in the clinic. Biomarkers are ideal choices for predicting preeclampsia during pregnancy. Attempts were made to study serum tumor necrosis factor α (TNF-α), C-reactive protein (CRP), vascular endothelial growth factor (VEGF) expression gene, endothelial nitric oxide synthase (eNOS), and p53 level in serum in the first trimester of pregnancy and biomarkers in the prediction of preeclampsia (Mervat et al., 2019).

As mentioned, one of the risk factors for the development of obstetric complications in women is a history of 3 or more births (multiparous women). The high incidence of anemia in multiparous women and the increase in the development of preeclampsia in the context of anemia, according to the literature, make us think about this and conduct research on these pregnant women.

To analyze the incidence rate of preeclampsia in multiparous women with iron deficiency anemia and its impact on the course of pregnancy and childbirth.

The study was conducted on 80 multiparous women with anemia, aged 18-44 years. In 19 (23,8%) of these women, preeclampsia was observed, 61 (76,3%) pregnant women did not have preeclampsia.

MATERIALS AND METHODS

The study was conducted on pregnant and postpartum women admitted to the maternity hospital No.5 (2014-2022) under the II Department of Obstetrics and Gynecology of AMU. The study is prospective in nature and consent was obtained from all patients. General blood analysis, biochemical analysis of blood were performed, coalograms were made, and anamnesis was recorded for each case carefully. Rates of HIV, RW, HCW, the classification, given by WHO experts, is based on the concentration of hemoglobin. Thus, mild, moderate, and severe anemia correspond to Hb concentrations, less than 100-110 g/l, 70-99 g/l, and 70 g/l, respectively.

Preeclampsia is classified as moderate or severe. HbsAg were determined and all pregnant women underwent the Ultrasound examination, Dopplerography, Cardiotocography of the fetus in the first and third trimesters.

Hypertension was diagnosed when the systolic blood pressure was ≥ 140 mm of mercury and the diastolic pressure was ≥ 90 mm of mercury. The result of + ≥1 (+ ≥0.3 g/l in daily urine) when protein was determined in the urine by the test-strip method confirmed proteinuria. Edema was observed in preeclampsia [Clinical protocol, 2012].

Regarding the age group of pregnant women, 34.4% of the women without preeclampsia and 15.8% of pregnant women with preeclampsia were 18-29 years old, while 65.6% of the women without preeclampsia and 84.2% of pregnant women with preeclampsia were 30-40 years old, $P_{\chi 2}$ =0.122.

When examining the social status of this group of women, it was detected that 75.4% of pregnant women without preeclampsia were housewives, 24.6% were working women, and 78.9% of pregnant women with preeclampsia were housewives and 21.1% were working women $P_{\chi 2}$=0.752.

Statistical analysis: The statistical integrity of the difference between the indicators of the groups was calculated in the SPSS-26 program with the Pt (Student-Bonferon), $P_{\chi 2}$ (Pearson), PU (Mann-Whitney) criteria (Gafarov 2022).

RESULTS AND DISCUSSION

The clinical characteristics of pregnancy and childbirth in women with preeclampsia were analyzed. First of all, special attention was paid to anamnestic data. Thus, in the anamnesis of the women without preeclampsia, abortion was not

registered in 32.8%, whereas 67.2% of the women had a history of abortion in preeclampsia cases, 26.3% of the women did not have an abortion, and 73.7% had an abortion ($P_{\chi 2}$=0.595). Abortion refers to spontaneous and artificial abortions. Gynecological diseases (infertility, genital inflammation, menstrual disorders, etc.) occurred in 3.3% of the non-preeclampsia pregnant women, and 15.8% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.049).

Extragenital diseases (cardiovascular pathology, obesity, varicose veins, etc.) were found in 18.0% of the pregnant women without preeclampsia and 31.6% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.208).

COA (complicated obstetric anamnesis) was present in 41.0% of the pregnant women without preeclampsia and in 73.7% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.013).

When analyzing the degree of anemia, the I, II, and III degrees were found in 17 (27.9%), 43 (70.5%), and 1 (1.6%) of the pregnant women without preeclampsia and 6 (31.6%), 11 (57.9 %), and 2(10.5%) in the pregnant women with preeclampsia, respectively ($P_{\chi 2}$=0.177) (Fig.).

MR (miscarriage risk) was 42.6% in pregnant women without preeclampsia and 52.6% in pregnant women with preeclampsia ($P_{\chi 2}$=0.444).

CFH (chronic fetal hypoxia) associated with the development of anemia was observed in 42.6% of the women without preeclampsia and in 68.4% of the women with preeclampsia ($P_{\chi 2}$=0.049).

FPI (Feto placental insufficiency) of the I, II, and III degrees was detected in 1.6%, 0.0%, and 1.6% of the pregnant women with preeclampsia and 21.1%, 0.0%, and 0.0% of the pregnant women without preeclampsia ($P_{\chi 2}$=0.008).

IUGR (Intrauterine growth retardation) occurred in 3.3% of the pregnant women without preeclampsia and in 21.1% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.008).

In cases without preeclampsia, the I degree birth weakness occurred in 24.6%, the II degree birth weakness in 6.6% of the women giving birth naturally, whereas in the pregnant women with preeclampsia, the I degree birth weakness occurred in 21.1%, and the II degree birth weakness in 5.3% of the women ($P_{\chi 2}$=0.922).

PROM (pre-labor rupture of membranes) was observed in 59.0% of the women without preeclampsia and in 26.1% of the women with preeclampsia ($P_{\chi 2}$=0.013).

PB (Premature birth) occurred in 9.8% of the pregnant women without preeclampsia and in 36.8% of the pregnant women with preeclampsia ($P_{\chi 2}$=0.005).

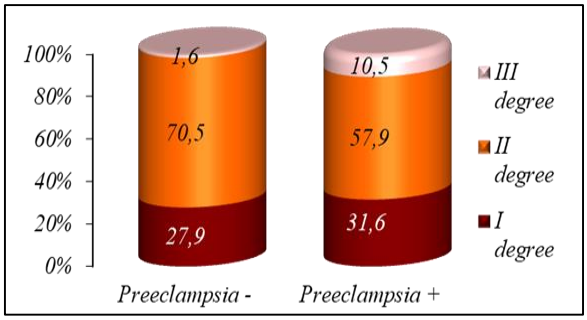

Fig. Frequency of anemia incidences

In cases without preeclampsia, 77.0 % of the women attended the hospital in the I period and 23.0% in the II period of pregnancy. Whereas, 89.5% of the patients with preeclampsia attended the hospital in the I and 10.5% in the II period of pregnancy ($P_{\chi 2}$=0.237).

Hysiological births and abdominal births occurred in 72.1% and 27.9% of women without preeclampsia, while in women with preeclampsia, this indicator amounted to 42.1% and 57.9%, respectively ($P_{\chi 2}$=0.017).

Regarding the fetus position during labor, in pregnant women without preeclampsia, 93.4% of the baby positions were head-down and 5.3% of them were breech. In patients with preeclampsia, 94.7% of the babies were in head-down and 5.3% breech positions ($P_{\chi 2}$=0.839).

Newborn babies were tested using the Apgar scale. At 1 min after birth for the babies delivered by the women without preeclampsia, Me=7.0 (7.0-7.0), and in the case of preeclampsia, Me=7.0 (7.0-7.0), (P_U=0.393) were obtained. At 5 minutes after birth, Me=8.0 (8.0-8.0) was obtained for the babies delivered by the women without preeclampsia.

The weight of newborns delivered by the women without preeclampsia was Me=3300.0 (3000.0-3600.0) and in the case of preeclampsia, it was Me=2800.0 (2400.0-3200.0), (P_U=0.001).

In terms of height, it was Me=51.0 (50.0-52.0) in cases without preeclampsia and 48.3 ± 0.9 in cases with preeclampsia P_F = 0.001, (P_U = 0.001).

As for the gestation period, it was Me=40.0 (38.0-40.0) in the pregnant women without preeclampsia, and Me=38.0 (36.0-39.0) P_U= 0.004 in the pregnant women with preeclampsia.

We observed a high incidence of iron deficiency anemia in multiparous women. According to the literature data, chronic iron deficiency anemia (80-95% of all anemias) is frequent in real practice in therapy [Turkhan D.I. et al., 2019].

Iron deficiency anemia adversely affects the course of pregnancy, childbirth, the postpartum period, the condition of the fetus and newborn. The main complications caused by iron deficiency anemia in pregnancy are the risk of miscarriage (20-42%); preeclampsia (40%); premature abruption of the placenta (25-35%); intrauterine growth retardation of the fetus (25%); premature births (11-42%) [Vavina O.V. et al., 2018]. Thus, in our study, the risk of miscarriage has increased significantly, and there has been intrauterine growth retardation of the fetus.

Severe preeclampsia is most common among pregnant women and results in an increased number of cesarean sections (Surina et al., 2019). In our study, cesarean sections have also been preferred.

In women with a history of spontaneous abortion, subsequent pregnancies may also lead to the development of preeclampsia (Sepidarkish et al., 2017). It should also be noted that the incidence rate of artificial or spontaneous abortions (COA) among multiparous women was also high in the current study.

Traffic, noise, and air pollution increase the risk of developing hypertension and preeclampsia during pregnancy (Pedersen et al., 2017). In this regard, we have studied the social status of pregnant women, and housewives were found to predominate among these women.

Early hospitalization and treatment of acute arterial hypertension with magnesium sulfate result in reduced mortality among pregnant women (Ramas et al., 2017). It is important to pay more attention to women with proteinuria during the prenatal period. Because it is important to study its development in preeclampsia, analysis and study of proteinuria should be performed to predict the development of preeclampsia (Chung et al., 2018).

Preeclampsia is a multifactorial and multisystem disease specific to pregnancy. It is the classic diagnosis of arterial hypertension and associated proteinuria after 20 weeks of pregnancy in women with normal prepregnancy blood pressure. Preeclampsia can also be considered in the absence of proteinuria with damage to target organs (Peracali et al., 2019). The long-term prevention of non-contagious diseases leads to a sharp reduction in the maternal and fetal risk of hypertension and preeclampsia.

Hypertension and preeclampsia are global health priorities and important issues for maternal and newborn health after non-infectious diseases, which are a global epidemic (Shakhbazova, 2018).

The presence of extragenital pathologies in pregnant women was found to be a risk factor for preeclampsia and eclampsia (Hannah et al., 2020). Based on our research, we suggested that anemia is a risk factor, as well as extragenital pathology for pregnant women with preeclampsia.

CONCLUSION

It has been established that preeclampsia is more common during pregnancy in multiparous women with iron deficiency anemia. Moderate and severe preeclampsia is more common in the II and III degrees of anemia. A study of the clinical and anamnestic data of these women showed that this condition was more common in women with complicated obstetric and gynecological anamnesis. Due to preeclampsia, cesarean delivery was predominant. Regarding the condition of the fetus, intrauterine chronic hypoxia and growth retardation became more common as the severity of preeclampsia and anemia increased.

Thus, it can be concluded that COA and CGA prophylaxis before pregnancy and effective contraceptive methods are needed. Preventing complications caused by anemia, performing timely diagnosis and quality antianemic treatment will ensure the health of the mother and fetus.

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Study of ornamental plants in parks and gardens of Absheron and Turkiye

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The article describes the results of research work on the study of the taxonomic composition and origin of ornamental trees, shrubs and herbaceous plants in the parks and gardens of Absheron and Turkiye, determining the number of trees, shrubs and herbaceous plants, forms of creating compositions, rules for grouping plants in compositions according to biological and decorative features, the use of small architectural forms, decorative forms of pruning trees and shrubs, the selection of promising species for landscaping, the prospects for using plants in various plantations. It was revealed that the studied plants adapt well to the conditions of Absheron and Turkiye, are promising and are recommended for the design of parks, gardens, squares, and the creation of various compositions.

Keywords: *Ornamental, plant, composition, landscape, introduction*

INTRODUCTION

In recent years, extensive work has been carried out in Absheron to develop green areas, create various compositions and improve the environment. In connection with the growth of cities and their newly created and changed structural plans, along with individual elements and territories, the entire urban environment is changing.

The rapid growth of housing construction in Baku and the reconstruction of the old part of the city's development significantly expand the boundaries of the capital of the republic. In this regard, the issue of greening the city, which should go in parallel with housing construction and its territorial growth, arises with all its urgency. According to the existing sanitary and hygienic standards in large industrial cities, including Baku, the area of green spaces should be 45-50% of the total living area, or 26-30 m^2 per inhabitant (Bochkova, 2017).

Ornamental tree and shrub plantations, flower beds and lawns determine the originality and beauty of cities and towns, and significantly improve the life of the population. Tropical plants introduced from the local flora and foreign countries, mainly from the Mediterranean countries, Holland, Turkey, Iran are widely used in the landscape design of Absheron when creating various compositions. Along with local plants, they enrich the parks, gardens, streets, squares, boulevards of Baku, contribute to the development of biodiversity and the rational recreation of people.

When designing, it should be remembered that in the end result, not only the materials and tools used by the designer but also the competent organization of the workspace play an important role (Vasilyeva et al., 2018).

Azerbaijan has accumulated vast experience in the field of architecture and urban planning. There is invaluable historical material on the development of landscape architecture over many centuries. During the reconstruction of existing and the formation of new cities of the republic, progressive ideas of an ecological urban planning approach to the planning and development of

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populated areas, the spatial organization of urban (developed) and natural (open) territories, and the harmonious relationship of architectural complexes and the natural landscape are being developed (Yeliseyeva, 2016).

Currently, the main task of landscape design is considered to be the creation of beauty and harmony in combination with the convenience of using the infrastructure of buildings, smoothing out the conflict between urbanization forms and nature, which often suffers from them (Konstantinova, 2018).

When creating flowerbeds and flower beds, one should try to make them not only beautiful from spring to autumn but also easy to maintain, and in no case should life be sacrificed for beauty (Kizima, 2015).

When planning the use of various tree species for landscaping, their functional purpose should be determined in advance. When planting in permanent places, it is necessary to once again clarify the features of the growth and development of trees so that they do not obscure windows, do not tightly cover balconies, loggias, terraces, arbors (Maksimenko and Maksimtsov, 2022).

Parks and gardens of Baku are the main green areas for mass recreation, walking and entertainment. In the conditions of Absheron, which do not have natural forests, these green areas are the main factors that improve the living conditions of the urban population and enrich the architectural appearance of the city. When planning and restoring the city of Baku, it is necessary to separate large areas for parks, gardens, squares (Shikanyan, 2018).

For the development of landscape design in Absheron, it is necessary to study the diversity of species and varieties of newly introduced tropical plants used in the landscaping of the republic, to create various forms of compositions using scientific methods. For this purpose, research work is being carried out in the laboratory "Landscape Architecture" of the Institute of Dendrology of the Ministry of Science and Education of the Republic of Azerbaijan. The purpose of the research work is to study the taxonomic composition and origin of ornamental trees, shrubs and herbaceous plants in the parks and gardens of Absheron, to determine the number of trees, shrubs and herbaceous plants, the forms of creating compositions, the rules for grouping ornamental plants in compositions according to biological and decorative characteristics, the use of small architectural forms, decorative forms of trimming trees and shrubs, the selection of promising plant species for use in landscape design, prospects for using plants in various plantations.

MATERIALS AND METHODS

The objects of research are various types and varieties of ornamental trees, shrubs and herbaceous plants. During the research work, various methods of foreign and local scientists were used. The taxonomic composition of ornamental plants was studied according to the method of Asgerov (2011), the forms of creating compositions, the rules for grouping ornamental plants in compositions according to the methods of Kizima (2015), Yeliseyeva (2016), Gulmammadova (2011) and Mammadov (2006).

RESULTS AND DISCUSSION

When conducting research work in 2019-2022, expeditions were organized on the territory of the National Seaside Park, the Philharmonic Garden, the Garden of Samad Vurgun, Khagani Park, Sahil Park and the Absheron Flower Park. In these parks and gardens, observations were made, herbariums were collected, the taxonomic composition and origin of ornamental trees, shrubs and herbaceous plants were studied, the number of trees, shrubs and herbaceous plants was determined, the forms of creating compositions, the rules for grouping plants in compositions according to biological and decorative characteristics, the use of small architectural forms, decorative forms of pruning trees and shrubs, the selection of promising species for landscaping, prospects for the use of plants in various plantations. The taxonomic composition of plants from 51 families, 78 genera and 97 species was studied in the National Seaside Park, 34 families, 45 genera and 55 species in the Philharmonic Garden, 23 families,

32 genera and 35 species in the Samad Vurgun Garden, 29 in Khagani Park families, 41 genera and 46 species, in the Sahil Park - 16 families, 26 genera and 28 species, in the Park of Flowers - 21 families, 29 genera and 37 species.

In the parks and gardens of Absheron, the creation of various forms of compositions in a regular style was studied - geometric shapes (rectangle, square, circle, rhombus, etc.) and in landscape or landscape style - original forms (flower garden, labyrinth, buta, etc.). Some forms of compositions studied in the parks and gardens of Absheron are shown in Figures 1-6.

The taxonomic composition and origin of the studied ornamental plants in the Park of Flowers on Absheron are shown in Table 1.

In the parks and gardens around the recreation areas, there are flower beds and palm trees, stone terraces are covered with climbing plants. Small architectural forms - fountains, lanterns, benches, sculptures, flower pots, pergolas create comfortable conditions for people resting here and enhance the artistic and architectural image of the park ensemble. Some small architectural forms studied in the parks and gardens of Absheron are shown in Figures 7-9. In geometric compositions, evergreen trees and shrubs are mainly planted in the center of the composition, and herbaceous plants are planted along the edges, and in compositions of the original form, 2 ornamental shrubs and herbaceous plants are mainly used, located in a free, landscape style, flower beds from annuals, biennials and perennials. When creating compositions, the combination of flower colors with each other, the height of the plants, the relationship to light, shade, moisture are taken into account. The plants are planted in such a way that other flowers bloom to replace faded flowers and the continuity of flowering is ensured. High plants are planted in the center of the composition, and low plants are planted along the edges so as not to obscure each other.

Geometric shapes compositions in a regular style

Fig. 1. Square shape

Fig. 2. Rhombus shape

Fig 3. Rectangle shape

Original forms of compositions in landscape or landscape style

Fig. 4. Decorative shape

Fig. 5. Flower shape

Fig. 6. Original shape

Table 1. The taxonomic composition and origin of the studied ornamental plants in the Park of Flowers on Absheron

Family	Genus	Species	Origin
1.*Pinaceae* Lindl.	1.*Cedrus* Mill.	1.*C.deodara* G.Don	Algeria
2.*Cupressaceae* F.W.Neger	2.*Juniperus* L. 3.*Thuja* (L.)Tourk. 4.*Cupressus* L.	2.*J.occidentalis* Hook. 3.*T.occidentalis* L. 4.*C.arizonica* Greene 5.*C.sempervirens for.horizontalis* L. 6.*C.x leylandii* A.B.Jacks. 7.*C.sempervirens* L.	Europe North America California Asia Minor Holland Asia Minor
3.*Magnoliaceae* J.St.Hil.	5.*Magnolia* L.	8.*M. fraseri* Walter	Japan
4.*Oleaceae* Lindl.	6.*Ligustrum* L 7.*Olea* L.	9.*L.japonicum* Thunb. 10. *L.vulgaris* L. 11.*O.europaea* L.	Caucasus, Ukraine Europe Australia
5.*Platanaceae* Dumort.	8.*Platanus* L.	12.*P.occidentalis* L.	North America
6.*Malvaceae* Juss.	9.*Hibiscus* L.	13.*H.syriacus* L.	China
7.*Rosaceae* Juss.	10.*Cotoneaster* Medic. 11. *Pyracantha* Roem. 12.*Photinia* Lindl. 13.*Prunus* Mill.	14.*C.lucidus* Schltdl. 15.*P.angustifolia* Franch. 16.*P.serratifolia* Desf. 17.*P.cerasifera* Enrn.	North Caucasus South China Asia, China Caucasus, Asia
8.*Celastraceae* Lindl.	14.*Euonymus* L.	18.*E.japonicus* Thunb. 19.*E.fortunei* L.	Asia Minor China
9.*Palmaea* Juss.	15.*Phoenix* L.	20.*P.canariensis* Chabaud.	Canary Islands
10.*Buxaceae* Dumort.	16.*Buxus* L.	21.*B.colchica* Pojark.	Asia, Caucasus
11.*Caprifoliaceae* Vent.	17.*Abelia* L. 18.*Lonicera* L. 19.*Viburnum* L.	22.*A.chinensis* R.Br. 23.*L.capriofolium* L. 24.*V.tinus* L. 25.*V.lucidum* L.	China Caucasus, Europe Europe Europe
12.*Asphodelaceae* Juss.	20.*Phormium* J.R.Forst.	26.*P.tenax 'Tricolor'* 27.*P.tenax 'Variegatum'*	New Zealand New Zealand
13.*Pittosporaceae* Lindl.	21.*Pittosporum* Thunb.	28.*P.tobira* Thunb. 29.*P.tobira 'Nana'*	South America Japan
14.*Punicaceae* Horan	22.*Punica* L.	30.*P.granatum* L.	Transcaucasia
15.*Elaeagnaceae* Lindl.	23.*Elaeagnus* L.	31.*E.commutata* Bernh.	Japan
16.*Berberidaceae* Torr.	24.*Nandina* Thunb.	32.*N.domestica* Thunb.	China, Japan
17.*Garryaceae* Lindl.	25.*Aucuba* Thunb.	33.*A.japonica* Thunb.	Japan, Taiwan
18.*Begoniaceae* C.Agardh.	26.*Begonia* L.	34.*B.cucullata* Willd.	South America
19.*Lamiaceae* Lindl.	27.*Teucrium* L.	35.*T.fruticans* L.	Mediterranean countries.
20.*Asteraceae* Bercht.	28.*Cineraria* L.	36.*C.maritima* L.	Mediterranean countries.
21.*Poaceae* R.Br.	29.*Leymus* L.	37.*L.arenarius* L.	Northern Europe
Total: 21 family, 29 genus and 37 species.			

Fig. 7. Decorative fountain

Fig. 8. Flower pot

Fig. 9. Street lamp

According to the agreement concluded between the Institute of Dendrology and the Department of Gardening of Baku City, the Institute of Dendrology studies the bioecological features of new trees, shrubs and herbaceous plants introduced from local and foreign flora, selects promising species adapted to local soil and climatic conditions and introduces them into the landscaping in Absheron. On the territories of parks and gardens of Absheron, the forms of trimming ornamental trees and shrubs were studied. Form pruning is carried out in order to preserve the natural forms of green spaces and give them artificial forms.

Safarov (1955) mentioned some of the features of *Pinus eldarica* Medw, which is drought-tolerant and does not require soil, is resistant to adverse environmental conditions, windy and salty conditions, and is indispensable in regions with difficult soil and climatic conditions. The use of this plant in the park, which is our research area, creates an advantage in terms of sustainability in landscape studies. It is grown because it is compatible with environmental conditions and adapts well.

Farzaliyev and Afonin (2016) said that *P.eldarica* is a non-frost resistant plant. It withstands single temperature drops to -22°C, but the isometric line of January temperatures of -3.5°C serves as the actual border of its distribution in Azerbaijan. Sufficient moisture must be provided for these plants to grow. Young plants can grow in Absheron in areas with a total annual precipitation of 200 mm, provided that periodic and supplementary irrigation is provided.

Natural woody and shrub species that can be used in landscape architecture have been determined in the province of Sivas in Turkey, and it has been reported that the *Juniperus communis* species can be among the plants that can be used in erosion prevention among the species that can easily grow on rocky and calcareous soils with strong root structure because Sivas has a mountainous and rough terrain (Bozkurt, 2021). In addition, researchers have included *J. communis* among the taxa that can be used in roadside and refuge vegetation. It can also be used for reforestation in mining areas. *J. communis*, which is among the plants that stand out in terms of aesthetic features such as flower, leaf, beauty of form, fruit, shadow effect, leaf color in autumn and creating a winter landscape, is also in our research area and gives beautiful images (Bozkurt, 2021).

According to Polat and Tunalıoğlu (2012), olive has dendrological properties such as visual quality value, color, texture, shape and size, and visual appeal, fascination, attractiveness, etc. It is one of the rare trees that can be examined with other parameters. The olive tree offers interesting views with its leaf, stem and flower and fruit colors. It creates interesting views that present the patterns of life according to the years with the patterns on its body like a history page and emphasize it with its branching shape. The arrangement of the leaves on the branches is interesting with the texture of the branches and stems. It is expected that the visual characteristics of the olive trees in Samed Vurgun Park will also come to the fore in this direction and have an aesthetic and functional impact.

The *Taxus cuspidata* plant is an interesting, dense shrub. It is a preferred species in landscaping, especially with its adaptations in cold and shaded areas. Low maintenance (USDA, 2022). The use of this species in Samed Vurgun Park draws attention. Sustainability is also ensured by the ease of maintenance.

According to Pignatti (1982), the plant *Platanus acerifolia* is a species with wide adaptation to various soil types, including strongly alkaline soils (Gratani et al., 2020). It is highly adaptable to various stress conditions along with pollution (Gratani and Varone, 2007; Pourkhabbaz et al., 2010). Because of such properties, it was widely used in gardens, irrigation channels and roadsides in England. It is known as the "London Plane" in England with its usage starting from the 1600s (Cennamo and Cafasso, 2002). It has started to be used in squares and boulevards in Italy (Pignatti, 2017). The shade of this plant species, which is also used in the park we researched, is used.

According to Ljubojević and Pušić (2022), *Hibiscus syriacus* provides a variety of ecosystem services, such as urban forestry, genetic resources, biodiesel production, erosion control, land reclamation, and plant breeding in public green areas. Due to its aesthetic features and rapid growth, it has become an ornamental plant that is

widely used in urban afforestation because it is preferred in the arrangement of public green areas (Vaz et al., 2017). *H.syriacus* provides soil stabilization and prevents landslides (Gomez-Baggethun and Barton, 2013). This decorative plant is used as a hedge plant and solitary in public green areas, due to the plant's mental and physical health-enhancing properties (Chan et al., 2012). The use of such a species in our park creates positive effects both aesthetically and functionally.

Similar to our study, in a study conducted in Ankara/Turkey, plant material was evaluated in city parks, public institution gardens, roadside trees and residential gardens in different districts (Aslan, 2020). In this context, *V.opulus* has been widely used in parks, official institutions and residential gardens, either as a single or as a group, in the places studied. Attention has been drawn to the use of this plant as an accent plant for its visual beauty with flower, fruit and autumn coloring. It is also mentioned that it can be a hedge plant. In our park, the plant has been used with the same design idea and adds beauty to the area.

Although mulberry trees are grown especially for silkworm (*Bombyx mori* L.), nowadays they are mostly used as ornamental plants in gardens, especially the delicious fruits of black mulberry, are grown for pharmacological and cosmetic use (Benedetta et al., 2007). Although mulberry trees are generally grown for their fruit, they are seen as shade trees in many parks and gardens. Plantings are available on roadsides and sidewalks. However, female mulberries are generally undesirable in urban areas. It creates pollution due to its fruits, especially in parking lots, cars and around the house. But recently, the use of edible fruit plants in living areas has become popular, and it is also used in some areas. *M.rubra* is used both for its shade and for nutrition because its fruits are a delicious species.

Buxus sempervirens plants grow best in moist, cool, loose, humus, and calcareous soils rich in plant nutrients. It is sensitive to winter frosts. It can be grown under trees, as they are shade-tolerant in moderate and humid climates. It is used alone, in groups, or as a hedge plant in parks and gardens. It can be used easily in shaded areas in gardens and parks. Since it is very suitable for pruning, it can be used as a hedge plant or topiary plant (MEGEP, 2016).

Euonymus japonica is not very picky in terms of soil. It can also be grown in soils with a high lime content. The plant grows in temperate and warm climates. Species that remain green in summer and winter are damaged by cold. It is used in groupings, fences, and curtain plants, or solitary. It is also suitable for growing in pots and on concrete floors such as roofs, balconies and terraces where there is no natural drainage (MEGEP, 2016). This plant has also been used in our research area in accordance with its purposes.

The *Ulmus minor* plant is a deciduous plant that loves full or partial sun. It is used as a filler and naturalizer in landscaping. It is a forest plant in natural-style gardens (Anonymous, 2022a). It is seen that this plant is used according to these criteria in the park we examined.

Laurus nobilis is widely preferred as a solitary and architectural plastic object in group compositions in green fence and curtain plant in landscaping applications due to its evergreen leaves and being highly suitable for pruning. It is also quite resistant to air pollution in cities. Again, since it is drought resistant, it can also be sed in landscaping applications in xeric areas (Pamay, 1971; Anşin and Özkan, 1993; Ürgenç, 1998). However, Yılmaz et al. (2006) stated that when using laurel in landscaping, due to the toxicity in its leaves and fruit, toxicity should be taken into account, especially in children's playgrounds and other green area arrangements (Ertekin et al., 2009). Compliance with these has also been observed in their use in our park.

The pale yellow flowers of *Pittosporum heterophyllum* are fragrant and there are 1-5 per cluster. It is used in all areas, from sunny to shade. The plant likes well-drained soils. It is used as a curtain or hedge plant in the landscape. It is tolerant of pruning. This type of use determined in Samed Vurgun Park has been evaluated in terms of use (Anonymous, 2022b).

CONCLUSION

As a result of research work in the laboratory "Landscape architecture" of the Institute of

Dendrology, Ministry of Science and Education of the Republic of Azerbaijan and Siirt University, Faculty of Agriculture, Department of Horticulture, Turkiye, it was revealed that tropical plants introduced from local flora and foreign countries in parks and gardens adapt well to the conditions of Absheron and Turkiye, are promising and are recommended for use in landscape design, in the design of parks, gardens, and squares in Absheron and Turkiye.

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Impact of prostaglandin analogues on the likelihood of macular edema development in a model of autoimmune uveitis against the background of ophthalmotonus pressure

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We conducted experimental tests to settle the debatable issue of whether the use of prostaglandin analogs in patients receiving them perioperatively during phacoemulsification might trigger macular edema. A model of steroid glaucoma was created in animals, and then, autoimmune uveitis was mimicked against the background of ophthalmotonus pressure - an inflammatory process that results in pathomorphological changes similar to those observed during phacoemulsification. Azarga and Taflotan were administered to create animal models against the above-mentioned background. These studies aimed to experimentally detect, in a comparable manner, the risk of developing macular edema in animals in the context of the administration of prostaglandin analogues, as well as to investigate the impact on the retina's vascular system. The studies found that Taflotan, when compared to Azarga, had a more noticeable negative effect on the diameter and quantity of functional capillaries in the retina against the background of the uveitis model. The administration of Azarga against the background of this model resulted in a decrease in the diameter of functional capillaries (despite an increase in their number), which exceeded the baseline values by just 2.6% (P=0.471). A reverse pattern was revealed with Taflotan administration. The functional capillaries in this group showed an increase in diameter (against the background of a decrease in their number), outperforming the intact capillaries by 15.9% (P=0.002). When used against the uveitis background, Taflotan administration raises the risk of macular edema by 26.7% (P=0.662), while Azarga decreases it by 8.3% (P=0.852). There is statistically no evidence to support the hypothesis that animals treated with Taflotan were more likely to develop macular edema than those treated with Azarga (P=0.442). Both of the tested medications do not raise the risk of getting ME in the glaucoma model. It can be concluded that Taflotan, in the absence of inciting conditions such as an inflammatory process, does not increase the likelihood of developing ME.

Keywords: *Macular edema, microcirculatory network, glaucoma, uveitis, prostaglandins, taflotan, azarga*

INTRODUCTION

Macular edema (ME) is a disorder when the macular region of the retina of the eye (RoE) becomes edematous due to an accumulation of excess fluid. ME manifestations can range from mild ones that hardly affect vision to severe ones that cause substantial visual impairment. Numerous factors can lead to ME, such as diabetic retinopathy, age-related changes, inflammatory illnesses, glaucoma, vascular abnormalities, including vascular occlusion, reticulopathy, etc. (Iftikhar et al., 2023). Prostaglandins (PG) are inflammatory mediators that play a crucial role in many areas of eye physiology and pathology, including the development of ME. PGs, particularly PGE2, have the ability to exacerbate inflammatory processes in vascular tissue, which may enhance the permeability

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of the vascular wall and cause fluid to build up in the macular region. PGs also play a role in blood flow regulation in RoE. Impairment of this control may lead to insufficient blood flow to the macular region or insufficient pumping of fluid from capillaries into tissues (Miyake and Ibaraki, 2002). Vascular growth factors, including vascular endothelial growth factor (VEGF), which is essential for the emergence of vascular alterations and ME, can be affected by PGs in terms of their intensity (Jiang et al., 2017). According to the results of some studies, taking PG medicines such as latanoprost and bimatoprost may help prevent the development of ME. This could be due to their capacity to enhance blood flow to the eye and reduce the intensity of vascular growth factors (Holló et al., 2020). Currently, research is being conducted to develop targeted treatments, including PG receptor inhibitors, which may offer novel approaches to the treatment of ME by inhibiting PG signaling pathways. These findings highlight the complex nature of the function of PG in the pathophysiology of ME and the possibility of utilizing PG medications in its treatment (Farnoosh et al., 2021, Urias et al., 2017). However, more research is needed to completely understand their mechanisms of action, as well as to determine their efficacy and safety.

Prostaglandin analogues (PGA), such as tafluprost, etc. are now routinely utilized in ophthalmology to lower intraocular pressure (IOP). These drugs have a convenient mode of administration with a satisfactory reduction in IOP in glaucoma. Nonetheless, other authors contend that PGAa might be the root cause of ME in patients with open-angle glaucoma (OAG) after cataract extraction (Phacoemulsification or PHACO) (Fakhraie et al., 2019).

We conducted experimental tests to settle the contentious issue of whether the use of PGAs in patients receiving them perioperatively during PHACO might trigger ME. A model of steroid glaucoma (Javadova, 2023) was created in animals, and then, autoimmune uveitis was mimicked against the background of ophthalmotonus pressure - an inflammatory process that results in pathomorphological changes similar to those observed during Phacoemulsification. The model obtained this way is similar to human iatrogenic glaucoma in many aspects, such as morphology and clinical features (Javadova, 2024). Next, the study drugs were instilled into the eyes of animals.

These studies aimed to experimentally detect, in a comparable manner, the risk of developing ME in animals in the context of the administration of PGAs, as well as investigate the impact on the retina's vascular system.

MATERIALS AND METHODS

The experiments were carried out on genus "chinchilla" rabbits, weighing 2.80-3.00 kg. In compliance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010, on the protection of animals used for scientific purposes, all experimental animals were housed in conventional vivarium conditions. The animals were reused in all cases where the experimental conditions allowed doing so.

The animals were divided into 10 groups:

The 1st group (3 rabbits, 6 eyes), included the rabbits in an intact state.

An intact animal model of experimental steroid glaucoma was modeled in group 2 (8 rabbits).

In group 3 (8 rabbits), Azarga, a fixed combination medication consisting of 1% brinzolamide and 0.5% timolol maliate, was instilled into the eyes of the animals against the background of 30-day experimental glaucoma.

In group 4 (8 rabbits), Taflotan (Santen, Japan), a PGA without preservatives containing 0.0015% tafluprost solution, was instilled into the eyes of the animals against the background of 30-day experimental glaucoma.

In group 5 (8 animals), the animals were sensitized by normal horse serum (NHS) against the background of experimental glaucoma.

The animals in group 6 (8 animals) were instilled with Azarga in their eyes after the sensitization by NHS.

The animals in group 7 (8 animals) were instilled with Taflotan in their eyes after the sensitization by NHS.

In group 8 (8 animals), the animals were sensitized by normal horse serum (NHS) against the background of experimental glaucoma. Then

they received a shocking dose of NLS intravitreally, resulting in uveitis.

The animals in group 10 (8 animals) were instilled with Azarga in their eyes against the background of uveitis.

The animals in group 10 (8 animals) were instilled with Taflotan in their eyes against the background of uveitis.

The experimental glaucoma model was created by administering one drop of 1% dexamethasone solution to each of the rabbits' eyes for 30 days. The long modeling time was chosen taking into account the fact, that persistent, irreversible glaucoma-related changes start to take place with prolonged use of steroids. This approach was chosen since it served the study's purposes, as the rabbits not only developed glaucoma but also cataracts. The model obtained this way is similar to human iatrogenic glaucoma in many aspects, such as morphology and clinical features (Javadova, 2023).

The uveitis model was developed as follows (Aksenova et al., 2017): to sensitize the animals, they were first subcutaneously injected with 5 ml of NHS. This was followed by a further intramuscular injection of 1 ml of NHS after 5 days. 9 days after the last injection, a shocking 0.07 ml dose of NHS was administered intravitreally into the right eye. The left eye was used as a control. The clinical presentation of uveitis started 3 days after the administration of the shocking dose in the right eye. Then the tested preparation was instilled into both eyes of the animals.

The animals underwent intravitreal visual inspection of their eyes, ophthalmological examination of the fundus and RoE, and measurement of intraocular pressure (IOP). All procedures were carried out with a manual ophthalmoscope. Blood for immunological testing and determination of the leukocytes, neutrophils, and lymphocyte count was drawn from the ear vein. Following the completion of the experiment, the eyeballs of the animals were enucleated under anesthesia (intravenous injection of 0.5-0.6 ml of ketamine), and micropreparations were prepared for morphological studies of RoE, for reviewing the state of the vascular bed, and the presence of ME. The IOP levels were measured with a portable Tono-Pen X pneumotonometer (Reichert, USA).

An ophthalmological examination of the anterior sector of the eye, fundus, and RoE was carried out using a Welch Allyn portable ophthalmoscope (USA, Dealmed). This equipment allows for analyzing the cornea, RoE, choroid (uvea), the state of the optic disc and optic nerve, optic disc exquamation, and ME.

The circulating immune complexes **(**CIC), immunoglobulins (Ig), and lymphocytes in the blood were evaluated using an enzymatic colorimetric approach on an FP-9019 analyzer (made in Finland), while the complement components were determined based on hemolytic activity. The leukocyte, lymphocyte, and neutrophil counts were determined on an Auto Hematology Analyzer Ratyo RT-7600 (China, 2019).

Morphometry of histological sections of RoE with a thickness of 4 microns was performed under a SCOP brand microscope (Holland), which gives an increase of X 400 and the possibility of automatic photographing of enlarged section images, following the method developed by G.G.Avtandilov (Avtandilov, 2002). This was accomplished using an ocular mesh installed on the microscope and a screw ocular micrometer. The following parameters were determined based on the obtained morphometry data:

1. The number of functional capillaries per 1 mm^2 area (pcs)
2. The diameter of functional capillaries per 1 mm^2 area (microns)
3. The total area of the microcirculatory network (sq. microns)
4. The total area of the medium-diameter arteries (sq. microns)
5. The lumen of the medium-diameter arteries (microns)

The acquired digital data were statistically analyzed using IBM Statistics SPSS-22 and MS EXCEL-2016 statistical tools for variational (U-Mann-Whitney) nonparametric analysis.

RESULTS AND DISCUSSION

Since the start of the rabbits' experimental glaucoma modeling, daily measurements of their intraocular pressure (IOP) have revealed a dynamic increase in IOP. This increase began on

the third day following the initiation of dexamethasone instillation and reached 20.4 mm Hg (maximum value of 21 mm Hg and minimum value of 19 mm Hg) from the initial 19.6 mm Hg intact value (IV) (maximum value of 20 mm Hg and minimum value of 19 mm Hg). On day thirty of the trial, a sustained rise in IOP of 54.1% (P=0.008) was noted, confirming the onset of experimental glaucoma.

On the 30th day, the instillation of Azarga and Taflotan as hypotensive agents resulted in a 37.4% decrease (P=0.002) and a 38.74% (P=0.02) decrease in IOP in the Azarga and Taflotan groups, accordingly, reaching the reference values in both groups.

A model of uveitis was created in the setting of ophthalmotonus pressure. The next day, after administering a shocking dose of NHS to the right eyes of animals, visual indicators of uveitis (clinical presentation) started to appear, which then worsened and reached a significant severity on the 3rd day. The examination of the cornea during this period revealed that all animals had 100% opacity, 100% corneal edema, 50% of cases had precipitates, 62.5% of cases had opacity on the anterior chamber, 25% of cases had hypopion, 43.75% of the total number of examined eyes had sporadic synechiae on the pupil, 12.5% of the total number of examined eyes had multiple synechiae; pupil occlusion was seen only in one eye and accounted for 6.25% of the total number of examined eyes. Also, vasodilation was observed in the iris in 3 eyes, which accounted for 18.75%, along with iris edema in 18.75%, and vitritis in 62.5% of the eyes.

Thus, the clinical presentation confirmed the development of uveitis in all animals.

Laboratory examinations of animal blood revealed (Table 1) that leukocyte content increased by 95.9% (P<0.001) and neutrophil content decreased by 22% (P=0.417) in animals against the background of sensitization by NHS without a shocking dose in the right eye (sample 2) compared with the indicators of animals with ophthalmotonus pressure before sensitization (sample 1). Following the injection of a stunning dose of NHS in the right eye (sample 3), markers of the animals changed in the following ways against the backdrop of NHS sensitization on day 3: The leukocyte count increased by 90.8% (P<0.001), and neutrophils by 105.8% (P<0.001).

The total hemolytic ability of the complement was determined, which was decreased by 84.4% (P<0.001) in the second sample and remained nearly at the same level (84.3%, P<0.001) in the third sample. The CIC content decreased by 99.1% (P<0.001) and 96.9% (P<0.001), respectively. At the same time, there was an increase of 120.5% (P<0.001) in T-lymphocyte content and 116.8% (P<0.001) in B-lymphocyte content in the second and third samples, respectively. The IgE blood levels increased more actively. IgE content in the 2nd sample increased 5.3 times (P<0.001), and in the 3rd sample, 6.5 times (P<0.001).

As the obtained results show, NHS, as an antigen, causes the generation of antibodies when injected subcutaneously. With repeated administration intramuscularly, an antigen-antibody response occurs, resulting in the formation of immune complexes with tissue affinity. The body is being sensitized. Blood levels of immune indicators increase. Administration of a shocking intravitreal dose leads to the development of an inflammatory process in the uveitis (Hsuan et al., 2021). As this takes place, the indicators of immune activation remain at a high level.

Table 1. Changes in immune parameters of the animal blood samples against the background of experimental glaucoma, sensitization by normal horse serum, and experimental uveitis

Index		Sample 1	Sample 2	Sample 3
	Valid N	**16**	**16**	**16**
Leukocytes 10^9/L	Mean ± Standard Error of Mean	7.07±0.04	13.85±0.47	13.49±0.19
	Minimum; Maximum	6.90; 7.50	10.20; 17.10	12.20; 14.80
	Median	7.00	14.20	13.70
	Percentile 25; Percentile 75	6.95; 7.15	12.65; 15.10	12.85; 14.05
	P_1		0.000	0.000
	P_2			0.291

Continued Table 1

Neutro-phils 10^9/L	Mean ± Standard Error of Mean	17.3±0.4	16.7± 0.5	35.6±0.4
	Minimum; Maximum	14.5; 20.1	12.1; 21.3	33.2; 38.1
	Median	17.1	16.7	35.6
	Percentile 25; Percentile 75	16.3; 18.6	15.8; 17.6	34.9; 36.5
	P_1		0.417	0.000
	P_2			0.000
Compli-мент с.u.	Mean ± Standard Error of Mean	7.07±0.04	1.10±0.02	1.11±0.02
	Minimum; Maximum	6.90; 7.50	1.00; 1.20	1.00; 1.20
	Median	7.00	1.10	1.10
	Percentile 25; Percentile 75	6.95; 7.15	1.00; 1.20	1.00; 1.20
	P_1		0.000	0.000
	P_2			0.841
CIC с.u.	Mean ± Standard Error of Mean	17.28±0.39	0.16±0.00	0.54±0.02
	Minimum; Maximum	14.50; 20.10	0.15; 0.18	0.40; 0.60
	Median	17.10	0.16	0.55
	Percentile 25; Percentile 75	16.30; 18.60	0.15; 0.17	0.50; 0.60
	P_1		0.000	0.000
	P_2			0.000
T- Lympho-cytes 10^9/L	Mean ± Standard Error of Mean	2.20±0.15	4.85±0.16	4.77±0.15
	Minimum; Maximum	1.20; 3.00	3.90; 5.90	3.90; 5.90
	Median	2.15	4.85	4.70
	Percentile 25; Percentile 75	1.75; 2.80	4.40; 5.15	4.40; 5.05
	P_1		0.000	0.000
	P_2			0.650
B – Lympho-cytes 10^9/L	Mean ± Standard Error of Mean	2.86±0.23	5.52±0.29	5.52±0.29
	Minimum; Maximum	1.40; 4.30	3.80; 7.30	3.80; 7.30
	Median	2.85	5.40	5.40
	Percentile 25; Percentile 75	1.95; 3.80	4.50; 6.50	4.50; 6.50
	P_1		0.000	0.000
	P_2			1.000
IgE кЕдА/л	Mean ± Standard Error of Mean	0.23±0.02	1.21±0.09	1.49±0.14
	Minimum; Maximum	0.00; 0.35	0.50; 2.17	0.56; 2.89
	Median	0.22	1.19	1.28
	Percentile 25; Percentile 75	0.19; 0.29	1.00; 1.30	1.20; 1.74
	P_1		0.000	0.000
	P_2			0.065

Sample 1 - against the background of experimental glaucoma, Sample 2 – against the background of sensitization by normal horse serum, and Sample 3 – against the background of experimental uveitis.

P_1 – the statistical significance of the differences as compared to Sample 1

P_2 – the statistical significance of the differences as compared to Sample 2

Wilcoxon Mann-Whitney Test

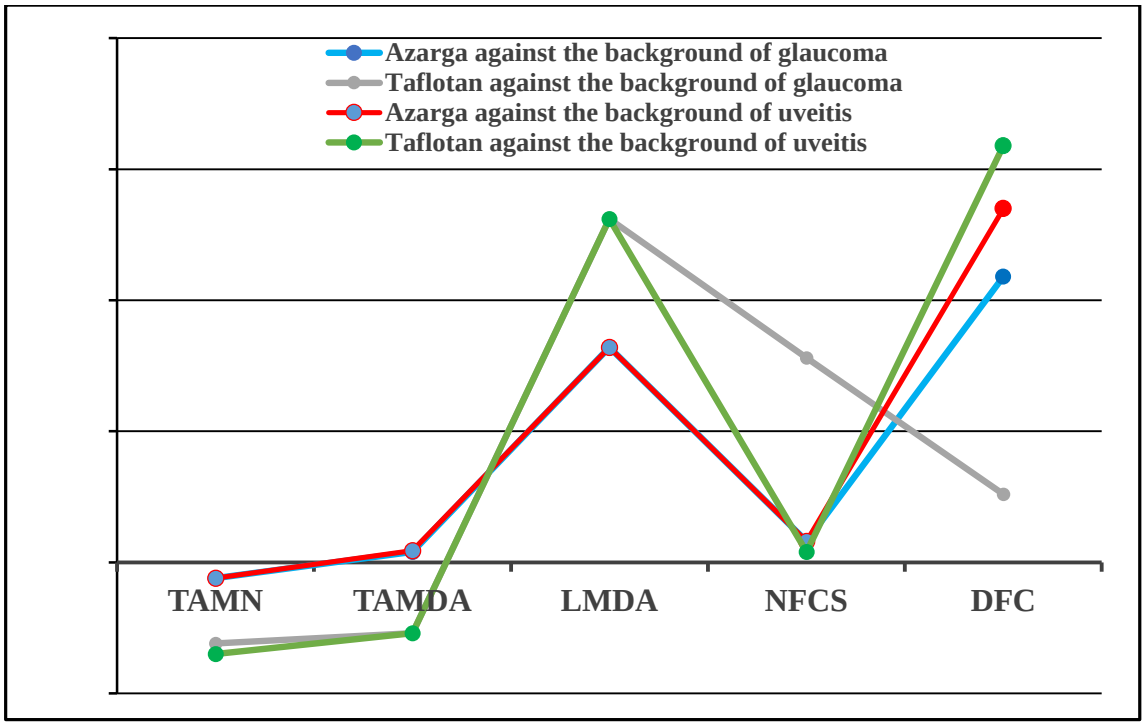

Fig. 1. Changes in the vascular bed of the retina of the eye caused by hypotensive agents (Azarga and Taflotan) against the background of an experimental glaucoma model and autoimmune uveitis in the setting of ophthalmotonus pressure.

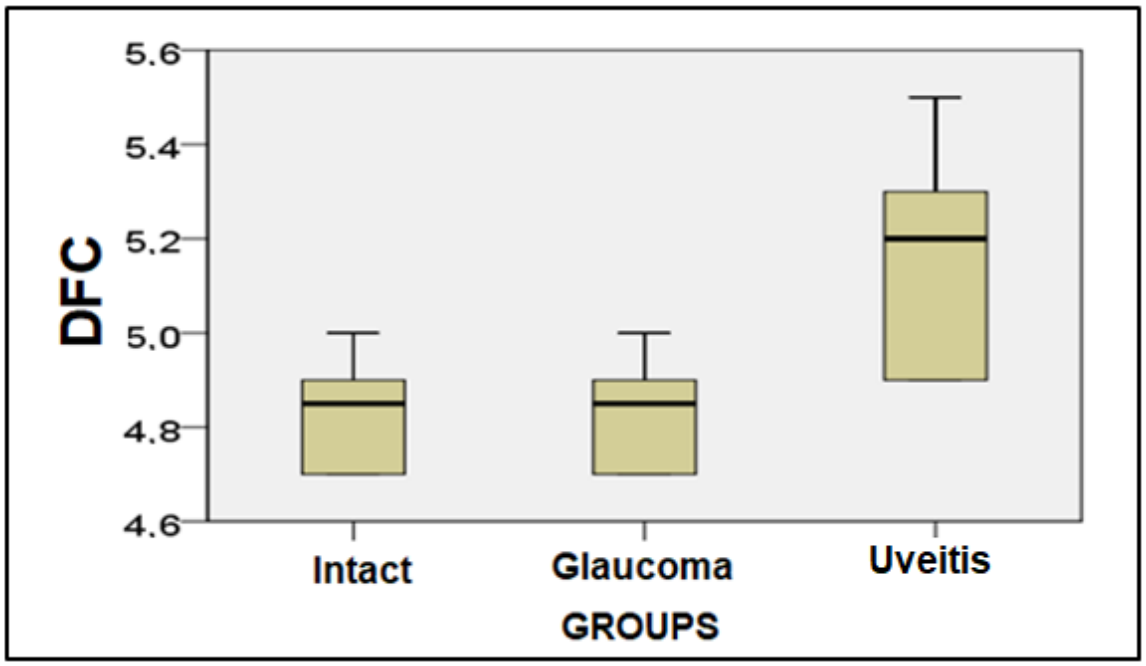

Fig. 2. Changes in the diameters of the functional capillaries of the retina of the eye with instillation of Azarga and Taflotan against the background of an experimental glaucoma model, and autoimmune uveitis in the setting of ophthalmotonus pressure.

The study of the changes in the vascular bed of RoE (Fig. 1) in the course of modeling autoimmune uveitis against the background of ophthalmotonus pressure showed that the total area of the microcirculatory network (TAMN) of RoE of the animals in which experimental glaucoma was modeled was reduced by 2.2% compared with IV (P=0.178). TAMN began to recover in animals treated with Azarga and Taflotan as a hypotensive drug against the background of the glaucoma model, and it remained at a level 0.6% less than IV throughout the trial period (P=0.641). In animals with the experimental autoimmune uveitis model against the background of ophthalmotonus pressure, this parameter is slightly higher than IV (by 0.3%, P=0.934). With the injection of the antihypertensive drugs into the eyes of animals against the backdrop of the uveitis model, the TAMN dropped by 3.1% (P=0.169) and by 3.5% (P=0.102) against the background of Azarga and Taflotan administration, respectively.

The total area of medium-diameter arteries (TAMDA) of RoE against the background of the glaucoma model, was slightly higher than IV (by 1.4% at P=0.681). TAMDA was decreasing in the animals receiving Azarga and Taflotan in their respective groups against the background of the glaucoma model, and it remained at a level 0.6% less than IV throughout the trial period (P=0.641). In the animals in which experimental autoimmune uveitis occurred against the background of ophthalmotonus pressure, TAMDA increased by 0.3% (P=0.806). The TAMDA dropped by 2.7% (P=0.292) in the animals whose eyes were instilled with Azarga against the backdrop of the uveitis model. Similar changes were taking place against the background of the Taflotan administration. In this group, TAMDA also decreased by 2.7% (P=0.292).

The lumen of the medium-diameter arteries (LMDA) increased by 6.5% (P=0.142) against the background of the glaucoma model. With the instillation of Azarga in the eyes of the animals with ophthalmotonus pressure against the background of the glaucoma model, the LMDA increased even more and exceeded the intact values by 8.5% (P=0.076), while in the animals treated with Taflotan under equal conditions, it increased by 8.2% (P=0.076). As it can be seen, the difference between the groups is negligible and statistically insignificant. As the presented data show, both studied drugs exert practically the same impact on LMDA against the steroid glaucoma model, resulting in a slight and statistically insignificant expansion of their lumen. However, changes were observed in all animals in both groups. Changes in the LMDA in the retina of the eyes of the animals upon modeling of autoimmune uveitis against the background of ophthalmotonus pressure were not detected. This indicator was also increased by 8.2% as compared to IV (P=0.120). Administration of the studied preparations in both groups led to even more increases in LMDA (by 13.1%, P=0.027).

The number of functional capillaries (NFCs) per 1 mm^2 (Fig. 2) of the RoE near the macular region against the background of an experimental glaucoma model diminished slightly (by 1.6%, P=0.809). The instillation of the studied antihypertensive agents in both groups led to a slight increase in NFC, where NFC exceeded IV by 0.8% (P=0.555). After modeling the autoimmune uveitis, NFC increased by 2.4% (P=0.411) (as compared to IV). In this case, the administration of Azarga increased NFC by 7.8% (P=0.097), and Taflotan increased NFC by 0.4% (P=0.052).

The diameter of functional capillaries (DFC) per 1 mm^2 area remained unchanged against the background of the experimental steroid glaucoma model. However, the administration of Azarga resulted in an increase in DFC in all animals by an average of 10.9% (P=0.026), and with Taflotan, this increase was 13.5% (P=0.004). Compared with IV, this indicator increased by 12.9% (P=0.022) against the background of the uveitis model. The administration of Azarga against the background of this model resulted in a decrease in DFC (in the setting of an increase in their number), which exceeded the IV by just 2.6% (P=0.471). A reverse pattern was revealed with the Taflotan administration. DFC in this group showed an increase (against the background of a decrease in their number), exceeding the intact capillaries by 15.9% (P=0.002).

The results of the macula examination in the eyes of rabbits for the presence of ME are shown in Table 2. The data presented in the table show that none of the six intact rabbits have experienced changes in the macula, and ME is absent in 100% of observations.

Table 2. Macular edema in the eyes of rabbits detected during the examination of the fundus with a manual ophthalmoscope

		Groups							
		Intact	**Glaucoma model**	**Glaucoma - sensitized**	**Uveitis**	**Sensitization – Azarga**	**Sensitization – Taflotan**	**Uveitis - Azarga**	**Uveitis - Taflotan**
ME is absent	Count	6	5	5	1	6	2	2	0
	Column N %	100.0%	83.3%	83.3%	16.7%	75.0%	25.0%	25.0%	0.0%
ME present	Count	0	1	1	5	2	6	6	8
	Column N %	0.0%	16.7%	16.7%	83.3%	25.0%	75.0%	75.0%	100.0%

In the eyes of animals, with the glaucoma model, ME was detected in one rabbit out of six examined, amounting to 16.7% (P=0.699) (according to the Mann-Whitney test results). The situation with the sensitized is similar, with ME detected only in one rabbit. Examination of the fundus of six animals with the uveitis model revealed the development of ME in five of them, amounting to 83.3% (P=0.015). In sensitized animals, ME was observed against the background of Azarga administration in two rabbits out of eight, which was 25% of all animals (P=0.491), whereas in animals treated with Taflotan, ME was detected in six animals, which was 75% (P=0.020). In the uveitis model rabbits, ME was detected in six animals (75%, P=0.020) against the background of Azarga administration, and ME was detected in all animals (100%, P=0.001) treated with Taflotan.

As it seems from the results, with the use of Taflotan against the background of sensitization and the uveitis model, as well as with the administration of Azarga against the background of uveitis, the development of ME is statistically confidently established in settings of uveitis.

Our research has demonstrated that not only the inflammatory process in the eyes (with uveitis) but also overall body sensitivity increases the probability of the development of ME. The administration of an AHP (Taflotan) in the animals sensitized by the NHS increases the likelihood of ME development.

Thus, the likelihood of developing ME against the background of experimental autoimmune uveitis is very high in all groups, and the sensitization of the organism by NHS also increases the likelihood of developing ME against the background of an AHP administration. That being said, the sensitization of rabbits by NHS against the background of an experimental glaucoma model does not affect the likelihood of ME development, with this likelihood not being statistically confirmed (P=1.000). However, hypotensive agents may provoke the development of ME in sensitized animals. Thus, with the instillation of Taflotan, a prostaglandin derivative, the likelihood of the development of ME is 58.3% higher as compared to sensitized animals, while with Azarga, this difference is 8.3% (P=0.852). It is evident that sensitized animals instilled with Taflotan have a 50% higher chance of developing ME than the animals injected with Azarga, but this likelihood is not supported by statistical evidence (P=0.105).

As previously demonstrated, when a shocking dose of NHS is administered into an animal's eye that has become sensitized, this is resolved by uveitis development, leading to an increase in the likelihood of developing ME up to 62.6% as compared to the unsensitized animals. However, such a difference, as defined by a percentage, is statistically insignificant. When used against the uveitis background, Taflotan administration raises the risk of ME by 26.7% (P=0.662), while Azarga decreases it by 8.3% (P=0.852). There is statistically no evidence to support the hypothesis that animals treated with Taflotan were more likely to develop ME than those treated with Azarga (P=0.442). Changes in the RoE microcirculation promote the effusion of fluid from the blood vessels, and because the RoE macular region is prone to ME because of its anatomical makeup, ME is mostly seen there (Mack et al., 2022, Yuan et al., 2024). Our study's findings support this once more.

Thus: 1. With the administration of dexamethasone to model glaucoma in rabbits, the animals developed a persistent ophthalmotonus pressure that did not dissolve after the termination of the administration of the preparation.

2. Hypotensive agents such as Azarga and Taflotan reduced IOP almost equally.

3. Instillation of Azarga and Taflotan against the background of an autoimmune uveitis model created by NHS in animals with ophthalmotonus pressure showed that these agents have an effect on the vascular bed of the retina of the eye, while statistically significant changes are observed with the administration of Taflotan. These changes consist mainly of the increase in DFC

4. Compared to Azarga, the administration of Taflotan in sensitized animals, and animals with experimental uveitis under ophthalmotonus pressure also resulted in considerably higher incidences of ME among the animals. However, despite the substantial percentage value of these indications, the data lack statistical reliability, making it impossible to state categorically that prostaglandin analogues may be the causes of ME in similar cases. It should also be noted that in the glaucoma model, neither Taflotan nor Azarga led to the development of ME in the animals.

CONCLUSION

According to the result of an experimental study, in response to the debatable question of the possibility of long-term AHP administration causing ME, it may be concluded that Taflotan, in the absence of provoking factors, for example, an inflammatory process, does not result in an increase in the likelihood of developing ME. Overall sensitization of the body also increases the likelihood of ME development. Therefore, in order to avoid an increased risk of developing ME during PHACO in patients with OAG combined with cataracts, it is recommended that for perioperative prescription of AHPs, along with other routine examinations, a blood count should be done to identify the patient's immune status.

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