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LECTINS AND THEIR APPLICATION IN FORENSIC MEDICAL PRACTICE

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ABSTRACT

The problem of obtaining economically cheap drugs for determining the group (in liquid form and in traces) is relevant. Diagnostic lectinology in relation to the determination of the blood group of the AB0 system is currently widely used in many countries of the world. The commercial cost of lectins is tens and even hundreds of times lower than the cost of rabbit absorption serum. The study of lectins in the seeds of some plants growing on the territory of Uzbekistan has shown the possibility of using them in the determination of antigens of the AB0 system.

Key words: agglutinins, agglutinogens, phytagglutinins, lectins, monoclonal antibodies, blood type, absorption of agglutinins.

INTRODUCTION

The examination of material evidence allows us to resolve many issues of investigative and judicial significance. Physical evidence includes weapons and objects that were used to commit a crime, stolen items, traces similar to blood, traces of human body secretions (saliva, semen, urine, sweat, etc.). Law enforcement agencies often find blood on the objects of the situation of the accident sites and seize it as material evidence. It contains a lot of information about the criminal or the victim, helps to identify the perpetrators by identifying the person by blood traces. Currently, forensic medical examination has the potential to establish a large number of group-specific factors in the blood and a number of other objects of biological origin. However, in forensic laboratories, blood tests for identity identification are performed mainly by antigenic differentiation within the AB0 system. For this purpose, expensive serums alpha, beta and heteroimmune serums anti-0, anti-A and anti-B are used, and sometimes separate sera series, which leads to great difficulties. The difficulty and high cost

of heteroimmune sera obtained by immunizing animals, as well as the moral responsibility in obtaining effective antibodies against some group isoantigens by actively immunizing people, sets the task of finding other more accessible ways to obtain antibody-like reagents. These searches are constantly relevant, as they are aimed at eliminating difficulties. During the period that followed from the second half of the last century, many plant species were studied in a number of countries in order to detect phytagglutinins.

Phytagglutinins (lectins) just as human and animal serum antibodies belong to the globulin fraction of proteins. Diagnostic lectinology, in relation to the determination of a person's blood group, is currently widely used in many countries of the world due to its availability, the commercial cost of which is tens or even hundreds of times lower than the cost of rabbit absorption serum. Therefore, the problem of obtaining economically cheaper drugs for determining the antigens of the AB0 system in the blood and secretions of the human body is urgent. Most phytagglutinins are polyagglutinins and to obtain reagent extracts specific to any antigen, they are processed in various ways. For example, by changing the technical conditions of extraction, diluting the extract with saline solution, changing the temperature regime, using micromolecular media, using sugars, absorbing the extract, using enzymatic red blood cells. The analysis of the literature shows the relevance and significance of studying the issue of identification of a person by traces of blood, secretions and tissue of the human body. The main and constantly decisive issue is the determination of the blood group of the AB0 system. Various ingredients are used for this: isohemagglutinating serums alpha and beta, heteroimmune serums anti-A, anti-B, anti-0, monoclonal antibodies anti-A, anti-B, as well as agglutinins of plant (phytagglutinins) or animal (protectins) origin. Each of these ingredients has its advantages and disadvantages in specific research cases. The introduction of new methods of analysis and the expansion of the volume of diagnostic reagents used increases the efficiency of forensic medical examination.

The use of monoclonal antibodies to determine the blood group of the AB0 system is an example of this. Although these antibodies have long been used in the fields of hematology, immunology, immunogenetics, human genetics, cell biology and tumors, but they began to spread in forensic medicine only at the end of the last century. Reagents based on these antibodies were used on a par with hemagglutinating and heteroimmune sera.

However, N. V. Mineeva and co-authors, when studying two blood samples, established a weak variant of blood group A, which did not give agglutination with monoclonal antibodies. Recently, the use of phytagglutinins has been widely

promoted in the practice of forensic medical examination of physical evidence to determine the blood type of the AB0 system. Perhaps this is due to the availability and cost-effectiveness of obtaining these reagents, the commercial cost of which is ten times lower than the cost of isoserum, immune and monoclonal antibodies.

V.O.Antonyuk fruitfully developing the problem of lectins, paid special attention to the biochemical aspect of their interaction with receptors. Antibody-like reagents of plant (lectins) and animal (protectins) origin are of extremely important forensic serological significance. In relation to the determination of human blood groups, diagnostic lectinology, which arose after the discovery of group-specific phytagglutinins, in its achievements has not only reagents to the antigens of the AB0 and MN system, but also Rhesus. In lectinology, the object of study and application is mainly the seeds of plants of the legume family. The advantage of this family, in addition to the rich protein composition of the seeds, is also that it is extensive, includes about 12,000 species and is cosmopolitan in terms of prevalence. Studies of the question of which parts of the plant contain hemagglutinins (lectins) have shown that they are more often found in seeds than in leaves, stems and roots. Most seeds contain them in the mass of the kernel. However, according to the instructions of Schmidt G, some, in particular, the seeds of Enonumis vulgaris contain them more in the shell. Lectins to the group-specific AB0 system in some cases occur in the form of only anti-A or anti-B; in others-anti-0+A or anti-0+B, and in others-anti-A, anti-B and anti-0 together. It is interesting to note that the presence of agglutinin-like substances in plants was established much earlier than the agglutinins of human and animal blood.

For example, in 1888, H. Stillmark found in castor seeds the presence of a substance capable of giving agglutination with human red blood cells, i.e. 13 years earlier before K. Landsteiner discovered the presence of agglutinins in human blood. Later, the works of a number of authors proved that substances that have the properties of antibodies against cells and certain fluids of the human body are found in many representatives of the plant and animal world. The appearance of agglutinins to agglutinogens of the AB0 system in plant seeds resembles their formation in humans. They appear in the process of maturation. At first, their number increases, and when the seeds germinate, it decreases. A person has the same thing. In a newborn, as a rule, they mature after a year, increase in adulthood and begin to decrease in old age. If we take into account the instructions of M. Krupe that some seeds of the same plant contain agglutinins, while others are devoid of them, then the same phenomenon can be observed in humans. For example, it has already been established that the presence of agglutinins is determined in human secretions (saliva, semen, etc.), and they are detected in some

people, and in others they are not determined. According to these properties of the human body, people can be divided into "excretors" and "non-excretors" of agglutinins.

In relation to the determination of the blood group of the AB0 system, diagnostic lectinology began to develop after the discovery of group-specific phytohemagglutinins by the scientist K.O. Renkonen. The author, studying the seeds of 99 species of motyl plants, found anti-A agglutinins in the seeds of Vicia cracca and anti-H (anti-0) in the seeds of lotus tetragonolobus, labrirnum alpinum, etc. Kgire also isolated protein substances from the seeds of Lotus tetragonolobus and Labrirnum alpinum, which gave agglutination of human red blood cells of group 0(H) in a titer of 1:256, and they did not interact with the red blood cells of horses, sheep, cows, rabbits, mice, guinea pigs and chickens. Studies of the question of which parts of the plant contain hemagglutinins have shown that they are more often found in seeds than in leaves, stems and roots. Most seeds contain them in the mass of the kernel. However, according to the instructions of G. Schmidt, some, in particular, the seeds of Eunonumis vulgaris contain them more in the shell. Kozlova-Lavrinenko found agglutinins in 8 of the 16 samples studied when studying seeds of wild plants. In 6 out of 8 cases, they were in the core mass and in 2 cases in the shell (seeds of Eunonumis planipes, Caragana qrufex). Among the plants studied by her, the most interesting were Vicia cracca and Cornilla varia, as containing isolated agglutinins of high titer.

In the study of 9 extracts of underground root plants-tubers of potatoes, carrots, onions, radishes, Jerusalem artichoke, gladiolus,canna and beet), agglutination of red blood cells of group A,B,0 did not occur in 6 of them, agglutination of red blood cells of group A,B,0 was observed in the remaining 3 (gladiolus, Jerusalem artichoke, patato). Potato tuber extract agglutinated red blood cells of all groups of the AB0 system, and the other two Jerusalem artichoke and gladiolus agglutinated red blood cells A and 0, B and 0, respectively. All three root plants-potatoes, Jerusalem artichoke and gladiolus-contained phytoagglutinins that do not have selectivity of action.

They had a less narrow specificity, reacted not with one but with two or three antigens. Titrations of extracts showed a high titer of phytagglutinin of potato tuber with all red blood cells (up to 1:1024) and a low titer in gladiolus and Jerusalem artichoke.

Material and research methods

Investigated dried fruits of herbaceous elderberry (Sambucus ebulus L), seeds of the plant (Chamaecytisus ruthenicus, Saphora japonica L, Phaseolus vulgaris Savi and of ten grape varieties). 198 experimental blood spots of living

individuals were studied (the first group ($0_{\alpha\beta}$) -98, the second group ($A\beta_0$) -46, the third group (B_α) -40 and the fourth group (AB0) -16).

To determine the presence of phytagglutinins in the fruits of herbaceous elderberry and seeds, extracts were prepared according to the method proposed by Prof. M. I. Potapov. To do this, the dried fruits and seman are crushed in a mortar, turning them into a homogenate, and poured with an isotonic solution of sodium chloride in a ratio of 1:10. After careful mixing of the ingredients, the resulting extract is kept in a thermostat at a temperature of $+37^0$ C for 3 hours, and then stored in the refrigerator for $+4\text{-}6^0$ C for 16-18 hours. After such extraction, they centrifuge and the nozzle part is filtered through a decontaminated paper filter. Thus, the prepared extract is stored at $+4\text{-}6^0$ C in a closed flask without the addition of antibacterial substances. The study is performed in the reaction of hemagglutination to human red blood cells of the AB0 system. The extracts are studied by the test tube method with an hour and a half contact with a 2% suspension of red blood cells (3 drops of liquid + 1 drop of suspension), followed by centrifugation for one minute at 1000-1500 rpm. The results of the reaction are taken into account with the naked eye and with the help of a microscope.

The determination of the blood group in spots is performed by the methods of agglutinin absorption and elution absorption.

Research results. In the study of extracts from the seeds of Saphora Japonica L. the presence of anti-B phytagglutinins was found both individually and in combination with other antibodies (B+0, B+A, B+A+0), but always with the predominance of anti-B. Studies of mature freshly obtained seeds of the Saphora japonica L. plant (collected in November 2019) growing on the territory of the city of Tashkent showed that, depending on the territorial location of the trees (eastern, western, northern, southern parts) they differ in the intensity of phytagglutinins, both individually (anti-B) and in combination with others (anti-B+0, anti-B+A+0), but always with a predominance of anti-B phytagglutinin.

In addition to underground root fruits, we investigated the presence and nature of phytagglutinins (lectins) in grape seeds growing on the territory of Uzbekistan, we studied the seeds of ten grape varieties. At the same time, the name given in the catalog of varieties of fruit, berry, citrus, nut crops and grapes was used. These grape varieties are: Nimrang, Parkent rozovy, Ak par, Khousaini Kelin Barmak, Khindogny, Sourkhak Kitabski, Gibrid Ranni Tcheurny, Kara Djandjal, Muskat Vengerski and Taifi Rozovy. The results of the study showed that phytagglutinins of various intensity were detected in the seed extracts of 9 grape varieties, and in one of them (Kaga Djandjal), agglutination of red blood cells of group A,B,0 was not observed. In 4 of the 9 extracts (Nimrang, Parkent Rozovy, Ak par, Khousaini Kelin Barmak), agglutination occurred with red blood cells of group A, and

agglutination did not occur with red blood cells of group B and 0. In three of these 9 extracts (Sourkhak Kitabski, Taifi Rozovy, Muskat Vengerski), agglutination was observed with red blood cells of group A and 0(H), and agglutination was not observed with red blood cells of group B. In two 9 extracts (Gibrid Ranni Tcheurny,Khindogny), agglutination with red blood cells of groups A, B and 0(H) occurred, at different intensities.

When checking the titer of 4 grape varieties Nimrang, Parkent Rozovy, Ak par, Khousaini Kelin Barmak) turned out to be different. Three of them (Ak par, Khousaini Kelin Barmak, Parkent Rozovy) had a titer equal to 1:8. The titer of Nimrang seed extract was 1:32.

In forensic biological laboratories, the determination of the blood group in spots is mainly performed by the methods of agglutinin absorption and elution absorption. If there is a sufficient amount of material, the method of absorption of agglutinins in quantitative modification is used. If there is not enough material, then an elution absorption reaction is used. To determine the antigen A in blood traces, the method of absorption of agglutinins in quantitative modification was used with phytagglutinin anti-A (seed extract grape «Nimrang») with a titer of 1:32.

We also studied the phytagglutinins of mature seeds of the plant Phaseolus vulgaris Savi, growing on the territory of Uzbekistan.

The study involved 8 samples of seeds of the genotype of the bean Phaeolus vigna catjanis - the concave part is colored black(1) and seven phenotypes of this bean Phaeolus vulgaris Savi - simple, rounded, variegated, pink-red(2); - rounded,variegated, dark red (3); - the concave part is pink-red(4); - simple ellipsoid with pink-red lines(5); - simple, white spherical shape(6); - simple ellipsoid, half red, half white(7); - simple, kidney-shaped, with pink dotted spots(8). As a result of the study, the presence of phytagglutinins was established in all samples of bean phenotypes (except for the bean genotype.- Rhaseolus vigna catjanis). Extracts of the phenotype of the seeds of Rhaseolus vulgaris Savi growing on the territory of Uzbekistan contain combined anti-A+B+0 phytagglutinins.

According to the intensity of agglutination of human erythrocytes and the titer height, the phytagglutinins differ from each other. At the same time, the best results were obtained in 3 out of 7 samples of Pseudolus vulgaris Savi (2,3,7). Checking the titer of all 8 samples of Phaseolus vulgaris (dilution in saline) showed different degrees of titer height of them. In the first sample, in which no phytagglutinins were detected in the undiluted extract, they were not detected in all dilutions. In the remaining seven cases, there was a well-expressed agglutination of

red blood cells of group A,B,0 to a sufficient degree of dilution. The presence of highly active phytagglutinin anti-A in the seeds of Phaseolus vulgaris Savi allows isolating it in a separate form with a titer of 1:16-1: 32 for practical use.

Dried fruits of herbaceous elderberry (Sambucus ebulus L) and broom seeds (Chamaecytisus ruthenicus) of 2016-2020 harvests growing in the city of Tashkent were studied. Extracts of herbaceous elderberry (Sambucus ebulus L) and broom (Chamaecytisus ruthenicus) have the property of agglutinating human red blood cells AB0 and contain anti-H phytagglutinins with different intensity. In the presence of phytagglutinins, their titer is determined. Titration of phytagglutinins is carried out by standard red blood cells of the same microdonors. The titer of these extracts turned out to be different: extracts from the fruits of herbaceous elderberry (Sambucusebulus L) had a titer equal to 1:64. The titer of broom seed extract (Chamaecytisus ruthenicus) was 1:48. Extracts from the fruits of herbaceous elderberry(Sambucusebulus L) and broom seeds (Chamaecytisus ruthenicus) were introduced into the reaction in dilutions of 1:16-1:32 for blood tests and with a titer of 1:32-1:64 for secretions. These extracts were used to determine the 0(H) antigen in blood stains.

198 experimental blood spots of living individuals were studied (the first group ($0_{\alpha\beta}$) - 98, the second group (A_{β}) - 46, the third group (B_{α}) - 40 and the fourth group (AB_0) -16). The antigens of blood spots were determined by the method of absorption of agglutinins in quantitative modification. As a result of the absorption of anti-H phytagglutinin under the influence of 98 blood spots of the first group, 52 of them showed a decrease in the titer of this phytagglutinin by 7-16 steps. In the remaining 36 cases, there was a decrease in the titer of anti-A phytagglutinin by 5-8 steps. The titer of this phytagglutinin under the influence of 36 carrier objects decreased by 1 step.

The carrier object of the remaining 72 blood spots did not affect the titer of anti-H phytagglutinin. Under the influence of 16 blood spots of group AB_0(IV), there was no decrease in the titer of anti-H phytagglutinin. The carrier of these blood spots, as well as 40 blood spots of the third group, did not affect the titer of anti-H phytagglutinin. The method of absorption of agglutinins in quantitative modification was also used to study spots formed by secretions (saliva, semen). In order to ensure the possibility of the most accurate comparison of the results of the study of various objects, blood samples and secretions of the same persons were introduced into the experiment and examined them simultaneously.

For example, blood, semen and saliva taken from the same men were examined. The obtained data showed that anti-H-Phytoagglutinin reacts differently with traces of different origins. Thus, the 0(H) antigen was detected much less

frequently and weaker in saliva than in blood, and this did not depend on the category of excretion of the subject and was observed even when examining fresh spots with small dilutions of the reagent. For example, detection was absent in some saliva spots of the $0_{\alpha\beta}$(I) group 1-2 weeks ago when they were examined with anti-H phytagglutinins. The 0(H) antigen was detected better in experimental semen spots than in blood spots. This, first of all, referred to those samples in which it was contained as a companion.

The severity of the reaction in some cases was also greater than with blood spots, while phytagglutinin anti-H reacted with samples of individuals belonging to the group of both "excretors" and "non-excretors". For discharge spots, the use of an extract with a titer of 1:32 was justified if the corresponding antigen in the spot was well expressed (including cases when the carrier object intensively absorbed the extract). In other cases, it is advisable to use an extract with a lower titer. The presence of a sufficient amount of the initial plant material and the positive results of expert testing suggest that extracts of herbaceous (Sambucusebulus L.) and broom seeds (Chamaecytisus ruthenicus) phytagglutinin anti-H can be applied to the practice of forensic medical examination in the study of very small traces of blood and secretions.

Thus, the conducted data allow us to develop rational ways of recommendations for improving the quality of forensic medical examination in the field of physical evidence in expert practice.

Conclusions

1. The properties and characteristics of lectins depend on the place of growth of the plant and the time of seed collection.

2. The presence of Saphora japonica L. in the seeds of the plant group specific anti-B phytagglutinins make it possible to use them in forensic medical practice when determining the blood group instead of isohemoagglutinating serums.

3. The presence of highly active phytagglutinin anti-A in the seeds of Phaseolus vulgaris Savi allows isolating it in a separate form with a titer of 1:16-1: 32 for practical use.

4. Extracts from the fruits of herbaceous elderberry (Sambucusebulus L) and broom seeds (Chamaecytisus ruthenicus) were introduced into the reaction in dilutions of 1:16-1:32 for blood tests and with a titer of 1:32-1:64 for secretions.

5. For discharge spots, the use of an extract with a titer of 1:32 was justified if the corresponding antigen in the spot was well expressed

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