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DETERMINATION OF THE GROUP AFFILIATION OF HUMAN HAIR IN FORENSIC BIOLOGICAL PRACTICE

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ABSTRACT

The results of the study showed that the extracts of grape seeds “Nimrang” and “Saphora Japonica L” can be used as hemagglutination drugs (anti-A and anti-B phytagglutinin) for the detection of antigens A and B in blood stains. The use of phytagglutinins is more economical than the use of expensive heteroimmune and isohemagglutinating serums in the detection of antigens A and B in blood stains during the examination of physical evidence. Detection of antigens A,B0(H) in hair by methods of absorption of agglutinins in quantitative modification, absorption-elution and affinity chromatography are more reliable and selected methods in expert practice for the study of physical evidence.

Key words: lectins, agglutinogens, agglutinins, blood type, phytoagglutinins, antibody titer, affinity chromatography.

INTRODUCTION

Currently, forensic medical examination has the potential to establish a large number of group-specific factors in 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 [1-14]. 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 [4,7].

The difficulty and high cost of heteroimmune serums obtained by immunizing animals, as well as the moral responsibility in obtaining effective antibodies against certain group isoantigens through active immunization of humans, sets the task of finding other more accessible ways to obtain antibody-like reagents. These searches are constantly relevant because they are aimed at eliminating difficulties. No less important is another fact that the study encourages scientists to also find antibody-like substances whose properties would differ from those of antibodies, but would contribute to deciphering the antigenic structure of the substance under study [5.10].

Since the second half of the last century, many plant species have been studied in a number of countries in order to detect phytagglutinins. However, the beginning of intensive development of a new field of non-infectious immunology - the science of lectins (agglutinins and precipitins of plant origin), a science that complements and expands classical immunology and begins to successfully compete with the latter in some areas became known in the 50s of the last century [2,8].

Phytagglutinins (lectins), as well as serum antibodies of humans and animals, belong to the globulin fraction of proteins. Diagnostic lectinology in relation to determining 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 of times lower than the cost of rabbit absorption serum. Therefore, obtaining economically cheaper drugs for determining blood group, both in liquid form and in spots, as well as in traces of secretions from the human body, is relevant [9].

The purpose of the study was to obtain specific lectins from extracts of seeds of some plants growing in Uzbekistan and use them in forensic practice.

Research objectives:

assessment and isolation of specific lectins in plant seed extracts (grape family “Vitis Vinifera L”, saphora “Saphora japonica L”, elderberry fruits “Sambucus ebulus L” and cytisus “Chamaecytisus ruthenicus”);

assessment of the titer and specificity of lectins by absorption method with erythrocyte antigens of the AB0 system;

assessing the possibility of using affinity chromatography of extremely diluted specific lectin to identify the corresponding antigen in a minimal amount of blood stain;

the use of phytaglutinins in determining the group identity of human hair.

The object of the study was 60 samples of liquid blood both of living persons and corpses, 100 samples of hair of living persons, 250 experimental stains and 435 stains of examination materials taken from the forensic biological laboratory of the Tashkent city branch of republican scientific-practical center of forensic medical examination.

Materials and methods of research. When obtaining and using phytaglutinins in forensic medical practice, the methods of absorption of agglutinins in quantitative modification, absorption-elution and affinity (biospecific adsorption) chromatography and the Schiff stain method were used to determine the group affiliation of liquid blood.

To determine the presence of phytagglutinins in plant seeds, extracts were prepared according to the method proposed by Professor M.I. Potapov (2003). To prepare extracts, the seeds were 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 thoroughly mixing the ingredients, the resulting extract is kept in a thermostat at a temperature of $+37^0$ for 3 hours, and then at 16-18 hours it is stored in a refrigerator at $+4\text{-}6^0$C. After such extraction, the mixture is centrifuged and the sediment is filtered through an ash-free paper filter. Thus, the prepared extract is stored at $+4–6^0$C in a closed flask without adding antibacterial substances. The study is carried out in the hemagglutination reaction to human red blood cells of the AB0 system. Extracts are studied in vitro with an hour and a half contact with a 2% suspension of erythrocytes (3 drops of liquid + 1 drop of suspension), followed by centrifugation for one minute at 1000–1500 rpm. The results of the reaction are recorded with the naked eye and using a microscope.

Results and discussion. Seed extracts from 10 grape varieties were studied to detect lectins (phytagglutinins). As a result of studying seed extracts of 10 grape samples, no phytagglutinins were found in one of them (Kara Djandal). In the remaining 9 cases, phytagglutinins of varying intensity were detected. In 4 out of 9 extracts (Nimrang, Parkent Rozovy, Akpar, Khousaini Kelin Barmak), agglutination predominantly occurred with red blood cells of group A. In 5 other cases (Sourkhak Kitabski, Taifi Rozovy, Muskat Vengerski, Gibrid Ranni Tcheurny, Khindogny) had a less narrow specificity, reacted not with one, but with two, three antigens of the AB0 system. When studying the titers of 4 grape varieties that detected phytaglutinin anti-A, three of them (Parkent Rozovy, Akpar, Khousaini Kelin Barmak) had a titer of 1:8, and the titer of the Nimrang seed extract turned out to be 1:64, we decided to use a colloidal medium (serum of the fourth - AB_0(IV) blood group) and received persistent anti-A phytaglutinin.

The hemagglutinating properties of extracts from the seeds of Saphora Japonica L were studied. Based on the fact that the territorial growing conditions of plants have a great influence on the presence and properties of phytagglutinins in seeds, we decided to study 20 samples of mature seeds of the plant "Saphora japonica L", collected (2023) directly from trees growing in the city of Tashkent, and from four parts (eastern, western, northern and southern parts of the city) (see Table 1).

Table 1

The degree of agglutination of erythrocytes with the extract obtained from the seeds of "Saphora Japonica L"

extract	Tashkent city regions											
	North			South			East			West		
	A	B	0	A	B	0	A	B	0	A	B	0
No cut	-	+++	-	-	-	-	-	+++++	-	-	+++	+
2	-	++	-	-	-	-	-	++++	-	-	+++	+
4	-	++	-	-	+++++	-	-	+++	-	-	+++	+-
8	-	+	-	-	++++	-	-	+++	-	-	++	-+
16	-	+	-	-	+++	-	-	+++	-	-	++	-
32	-	+-	-	-	+++	-	-	++	-	-	+	-
64	-	-+	-	-	++	-	-	++	-	-	+	-
128	-	-	-	-	++	-	-	+	-	-	+-	-
256	-	-	-	-	++	-	-	+-	-	-	-+	-
512	-	-	-	-	+	-	-	-+	-	-	-	-
1024	-	-	-	-	+-	-	-	-	-	-	-	-
2048	-	-	-	-	-+	-	-	-	-	-	-	-

Note: "++++" – large petal agglutination; "+++" – sand-like agglutination visible to the naked eye; "++" – agglutination visible to the eye in the form of conglomerates of various sizes; "+" denotes the gluing of all red blood cells into conglomerates of various sizes; "+-" – corresponds to small conglomerates against the background of a large number of non-glued red blood cells; "-+" – corresponds to small agglutinates of 3-5 glued red blood cells against the background of the majority of non-glued ones; "–" – indicate the complete absence of agglutination.

As a result of the study, phytagglutinins were found in all 20 extracts of the Saphora japonica L plant, and in 10 cases all phytagglutinins anti-A, anti-B and anti-0 were identified with a predominance of anti-B. In 8 out of 20 samples, only anti-B phytagglutinin was detected, in the remaining 2 cases anti-B phytagglutinin in combination with anti-0 phytagglutinin. It should be noted that specific isolated

anti-B phytagglutinin was mainly detected in the seeds of plants growing in the southern part of the city (only anti-B phytagglutinin was found in all 8 samples). Although in other cases phytaglutinin anti-B was detected in combination with anti-A or anti-0+anti-A, the titer of phytaglutinin anti-B was always high and ranged from 1:256 to 1:2048.

Extracts from the fruits of the herbaceous elderberry “Sambucus ebulus L” and the seeds of the Cytisus “Chamaecytisus ruthenicus” growing in the city of Tashkent were studied to identify phytagglutinins (lectins) in them. The data obtained showed that elderberry and Cytisus extracts have the property of agglutinating human erythrocytes AB0 and contain anti-H phytagglutinins with varying intensities. If phytagglutinins are present, their titer is determined; extracts from elderberry fruits had a titer of 1:64 and the titer of Cytisus seed extract was 1:48. Extracts from the fruits of the herbaceous elder “Sambucus ebulus L” and Cytisus “Chamaecytisus ruthenicus” were introduced into the reaction in dilutions of 1:16 - 1:32 for blood tests and a titer of 1:32 -1:64 for secretions.

It should be noted that extracts from plants (grape “Nimrang”, saphora “Saphora japonica L”, elderberry “Sambucus ebulus L” and Cytisus “Chamaecytisus ruthenicus”) have a number of advantages (high titer, specificity and ability to be absorbed, stability of characteristics, cost-effectiveness) and therefore can be recommended for practical use in forensic laboratories for the study of material evidence of biological origin.

Data from studies indicating that the sensitivity of the absorption-elution reaction, agglutinin absorption in quantitative modification and affinity chromatography showed that antigens A and B in blood spots can be detected by these methods using lectins. 60 experimental blood stains of living persons were studied ($0_{\alpha\beta}$ (I) – 10, A_{β} (II) – 22, B_{α}(III) –22 and AB_0(IV) – 6) (see Table 2).

Table 2

Results of the study of experimental blood stains

blood type	number of cases	the degree of reduction in the titer of FAa-A extract in the degree of absorption		degree of decrease in serum titer α in absorption steps		the degree of reduction in titer of FAa-B extract in absorption stages	
		blood stains	career objective	blood stains	career objective	blood stains	career objective
A_{β}	10 12	5-6 3-4	1 0	3-5 3-4	0 0	0 0	0 0
B_{α}	22	0	0	0	0	5-6	0
$0_{\alpha\beta}$	10	0	0	0	0	0	0
AB_0	6	3-4	0	3-4	0	4-5	0

As a result of the absorption of anti-A phytagglutinin under the influence of 22 blood spots of the A_{β}(II) group, in 10 of them a decrease in the titer of this phytagglutinin by 5-6 steps was observed. In the remaining 12 cases, a decrease in the anti-A phytagglutinin titer by 3-4 steps was observed. Under the influence of 6 blood spots of AB_0(IV) group, a decrease in the anti-A phytagglutinin titer by 3-4 steps was observed. The carrier object of these blood spots, as well as 22 blood spots of group B_{α}(III), had no effect on the anti-A phytagglutinin titer. Parallel control studies of the "α" serum titer under the influence of 22 blood spots of the A_{β}(II) group decreased by 3-5 steps. Under the influence of six blood spots of AB_0(IV) group, the α serum titer decreased by 3-4 steps, and the carrier object did not affect the titer of this serum. The titer of serum "α" and anti-A phytaglutinin did not change under the influence of 10 blood stains of group $0_{\alpha\beta}$(I) and the objects that carried these stains.

When studying the results of absorption of anti-B phytagglutinin under the influence of 22 blood spots of B_{α}(III) group, a decrease in the anti-B phytagglutinin titer by 5-6 steps was observed. The carrier object had no effect on the anti-B phytagglutinin titer. Under the influence of 6 blood spots of AB_0(IV) group, the phytagglutinin anti-B titer decreased by 4-5 steps, the "β" serum titer decreased by 3-4 steps. Under the influence of 6 blood stains, the AB_0(IV) group of carrier objects did not change.

Using the extract of "Sambucus ebulus L" to determine the 0(H) antigen in traces of blood by the absorption method of agglutinins in a quantitative modification, 200 experimental blood spots of living individuals were studied (first group ($0_{\alpha\beta}$) -98, second group (A_{β}) -46, third group (B_{α}) -40 and the fourth group (AB_0) -16). As a result of the absorption of anti-H phytagglutinin under the influence of 98 blood spots of the first group, in 62 of them a decrease in the titer of this phytagglutinin by 8-16 steps was observed. In the remaining 36 cases, a decrease in the anti-A phytagglutinin titer by 5-8 steps was observed. The titer of this phytagglutinin, under the influence of 36 carrier objects, decreased by 1 step. Under the influence of 16 blood spots of AB_0(IV) group, no decrease in anti-H phytagglutinin titer was observed. The carrier object of these blood spots, as well as 40 blood spots of group B_{α}(III), had no effect on the anti-H phytagglutinin titer.

In order to study the properties of the extract of "Nimrang" and "Saphora japonica L" for the determination of antigens A and B in traces of blood, 60 blood spots were studied using affinity chromatography ($0_{\alpha\beta}$(I) – 10, A_{β}(II) – 22, B_{α}(III) – 22 and AB_0(IV) – 6). In this case, along with iso-sera α and β, a little boat filled with extracts of Nimrang grape seeds and Saphora japonica L was placed at the bottom of the chromatographic chamber. Accordingly, a sheet of paper with

stripes, with the inscription “FAa-B”, “FAa-A”, meant phytagglutinins anti-A and anti-B. As a result of the study of 60 experimental blood spots in 22 blood spots of the A_{β}(II) group, anti-A phytagglutinin and α isoserum were found in the eluates. In the eluates of 22 blood stains of B_{α}(III) group and objects of their carriers, α agglutinin and anti-A phytagglutinin were not detected. In a study of 10 blood stains of the $0_{\alpha\beta}$(I) group, anti-A and anti-B phytagglutinins, as well as α and β agglutinins, were not detected in the eluates. In the study of 6 blood stains of AB_0(IV) group, phytagglutinins anti-A and anti-B, as well as agglutinins α and β were found in the eluates of blood stains. Agglutinins α and β, as well as phytagglutinins anti-A and anti-B, were not detected in the eluates of objects carrying these stains.

50 hair samples taken from the heads of living persons were also examined. Determination of antigens of the AB0 system in hair was carried out by the absorption method of agglutinins in a quantitative modification: according to group affiliation, 8 hair samples belonged to the first ($0_{\alpha\beta}$); 16 to the second (A_{β}); 16 to the third (B_{α}) and 10 to the fourth (AB_0) blood groups. These studies used extracts of Nimrang grape seed, Sophora japonica L, Sambucus ebulus L and Sambucus ebulus L to determine the grouping of human hair. When absorbing the anti-A phytaglutinin titer under the influence of 16 hair samples of the second group, A_{β}(II) decreased by 4-5 steps. Under the influence of 10 hair samples from the AB_0(IV) group, a decrease in the anti-A phytaglutinin titer by 3-4 steps was observed.

The anti-A phytagglutinin titer did not change under the influence of 16 hair samples of the third group B_{α}(III) and six hair samples of the first group $0_{\alpha\beta}$(I). Parallel control studies of alpha serum titer under the influence of 16 hair samples from the second group decreased by 3-5 steps. Under the influence of 10 hair samples from AB (IV) group, the alpha serum titer decreased by 3-4 steps. Also, the alpha serum titer under the influence of 16 hair samples of the B_{α}(III) group and six hair samples of the $0_{\alpha\beta}$(I) group did not change. When absorbing the anti-B phytaglutinin titer under the influence of 16 hair samples, the Bα(III) group decreased by 4-5 steps, and the titer of beta serums decreased by 3-5 steps. Under the influence of 10 hair samples of the AB0(IV) group, the anti-B phytagglutinin titer decreased by 4-5 steps, and the beta serum titer decreased by 3-4 steps. The titer of anti-B phytaglutinin and β serums did not change under the influence of 16 hair samples from the Aβ(II) group and six hair samples from the $0_{\alpha\beta}$(I) group.

When determining the group affiliation of human hair using the absorption-elution method, 12 hair samples from males aged 25–55 years were studied. In these studies, extracts of grape seed “Nimrang”, saphora “Sophora japonica L” and

elderberry fruit “Sambucus ebulus L” were used to determine the grouping of human hair. The results of the study showed that under absorption conditions of +48–52^{0}C, time 30 minutes with different series, antigen A was detected in 3 hair samples, these hairs belonged to persons with the second A_β(II) blood group. Antigen B was detected in 4 hair samples - these hairs belonged to persons with the third B_α(III) blood group, antigens A and B were detected in 3 hair samples, these hair samples belonged to persons of the fourth AB0(IV) blood group and antigens H were detected in 1 hair sample, the hair belonged to persons of the first $0_{\alpha\beta}$(I) blood groups.

To determine the group affiliation of human hair, 52 hair samples taken from 25 men were studied using affinity chromatography (see Table 3).

Table 3

The results of research on the group affiliation of human hair

Group	Number of hair samples	Degree of agglutination of eluted antibodies by erythrocytes										positive (+)	Negative (-)
		Phytagglutini anti-A					Phytagglutini anti-B						
		+++	++	+	±	-	+++	++	+	±	-		
A	20	2	9	7	2						20	20	
B	22					22	3	9	9	1		21	1
0	10					10					10		

As can be seen from Table 4, in 20 hair samples belonging to individuals with the A_β(II) blood group, in two of them in the eluates of chromatographed hair follicles, the most intense pattern of the “+++” reaction was observed with the membrane. In 9 out of 20 objects, the appearance of medium agglutinates was noted, containing 5–7 erythrocytes against the background of freely lying erythrocytes in the field of view. They were designated by the sign “++”. In 7 cases, small “+” agglutinates were observed in the field of view of the microscope, containing 3-4 erythrocytes against the background of many freely lying erythrocytes. And finally, in the remaining 2 out of 20 cases, single small agglutinates were observed, containing 2-3 red blood cells against a background of solid free-lying red blood cells in the field of view, which we designated as doubtful cases with the sign “±” and could not be attributed to positive results .

Experimental studies of 22 plucked hairs of people with B_{α}(III) blood group, in 21 cases gave clearly positive results (in 3 cases "+++" was noted, in 9 cases – "++" and in 9 cases – "+"), and in one case a questionable result was obtained, which was considered as a negative result. In all cases, nonspecific binding was not noted, that is, in the eluates of 22 hairs pulled out from this group, agglutination with group A erythrocytes did not occur. It also did not occur with erythrocytes A and B in the eluates of 10 pulled out hairs of people with $0_{\alpha\beta}$(I) blood group. Thus, when studying the group affiliation of hair, the detection of antigens A, B 0 (H) in hair by methods of absorption of agglutinins in quantitative modification, absorption-elution and affinity chromatography are more reliable and selected methods in expert practice for the study of material evidence.

CONCLUSION

1. In extracts of plant seeds (grape "Vitis Vinifera L", saphora "Saphora japonica L", fruits of elderberry "Sambucus ebulus L" and broom "Chamaecytisus ruthenicus") growing in Uzbekistan, lectins to the erythrocyte AB0 systems were found and the possibility was studied detection of antigens A, B, 0(H) in blood stains in the practice of forensic medicine.

2. The specificity of lectins, the titer of phytagglutinins anti-A, anti-B and anti-H were determined by the absorption method of agglutinins in a quantitative modification with erythrocyte antigens of the AB0 system. Phytagglutinin anti-A (Nimrang extract) titer 1:64; anti-B phytagglutinin (Saphora Japonica L extract) titer 1:256–1024; anti-H phytagglutinin (Chamaecytisus ruthenicus extract) titer 1:48; the anti-H phytagglutinin titer (Sambucus ebulus L extract) was 1:64.

3. Extremely diluted anti-A, anti-B and anti-H phytaglutinins will allow the use of affinity chromatography to identify the corresponding antigens in a minimal amount of blood stain. Determination of group membership in human hair, antigen A with extracts of grape seeds "Nimrang", antigen B with extracts of saphora seeds "Saphora japonica L" and 0(H) with extracts of elderberry fruits "Sambucus ebulus L" served as the basis for its use in antigenic assessment.

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