YEVROSIYO PEDIATRIYA AXBOROTNOMASI/ЕВРАЗИЙСКИЙ ВЕСТНИК ПЕДИАТРИИ

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THE USE OF SEED EXTRACT "SAPHORA JAPONICA L" AND GRAPES "NIMRANG" FOR THE DETERMINATION OF ANTIGENS OF THE AB0 SYSTEM IN BLOOD TRACES

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Resume

The results of the study showed that extracts of Nimrang grape seeds and Japanese saphora can be used as hemagglutination preparations (anti-A and anti-B phytagglutinin) in detecting A and B antigens in blood stains. In the study of 60 experimental blood spots by the method of absorption of agglutinins in a quantitative modification, phytagglutinin anti-A gave positive results only with antigen A, and phytagglutinin anti-B with antigen B in preserved blood spots B. The use of phytagglutinins is more economical than the use of expensive heteroimmune and isohemagglutinating sera when detecting antigens A and B in blood stains in the study of material evidence.

Keywords: lectins, agglutinogens, agglutinins, blood group, phytagglutinins, antibody titer.

ИСПОЛЬЗОВАНИЕ ЭКСТРАКТА СЕМЯН “SAPHORA JAPONICA L” И ВИНОГРАДА “NIMRANG” ДЛЯ ОПРЕДЕЛЕНИЯ АНТИГЕНОВ СИСТЕМЫ АВ0 В СЛЕДАХ КРОВИ

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Резюме

Результаты исследования показали, что экстракты семян винограда Нимранг и сафоры японской могут быть использованы в качестве препаратов гемагглютинации (фитаглютинина анти-А и анти-В) при выявлении антигенов А и В в пятнах крови. При исследовании 60 экспериментальных пятен крови методом абсорбции агглютининов в количественной модификации фитагглютинин анти-А дал положительные результаты только с антигеном А, а фитаглютинин анти-В с антигеном В в сохраненных пятнах крови В. Применение фитагглютининов более экономично, чем применение дорогостоящих гетероиммунных и изогемагглютинирующие сыворотки при выявлении антигенов А и В в пятнах крови при исследовании вещественных доказательств.

Ключевые слова: лектины, агглютиногены, агглютинины, группа крови, фитагглютинины, титр антител.

ҚОН ДОҒЛАРИДА АВ0 ТИЗИМИНИНГ АНТИГЕНЛАРИНИ АНИҚЛАШ УЧУН “SAPHORA JAPONICA L” ВА “NIMRANG” УЗУМ УРУҒЛАРИ ЭКСТРАКТИНИ ҚЎЛЛАШ

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Резюме

Тадқиқот натижалари қон доғларида А ва В антигенларни аниқлашда “Nimrang” узум ва “Saphora Japonica L” сафора уруғи экстрактларидан гемагглютинацияловчи препаратлар (фитагглютинин анти-А ва анти-В) сифатида қўлланилиши мумкинлигини кўрсатди. Агглютининлар абсорбцияси миқдорий модификация усулида 60 та эксприментал қон доғларини текшириш жараёнида фитагглютинин анти-А фақатгина антиген А ва фитагглютинин анти-В эса антиген В сақланган қон доғларида мусбат натижалар берди. Ашёвий далиллар экспертизасида қон доғларида А ва В антигенларини аниқлашда қиммат нархли гетероиммун ва изогемагглютинацияловчи зардобларни қўлланилишига нисбатан фитагглютининларни қўллаш иқтисодий жиҳатдан анча арзондир.

Калит сўзлар: лектинлар, агглютиногенлар, агглютининлар, қон гуруҳи, фитагглютининлар, антитаналар титри

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Relevance

Examination of traces of blood is the main part of the work of forensic medical examination of physical evidence. It can become an objective confirmation of the crime committed. Traces of blood, or, as they are called "silent witnesses of the crime", contain information about the offender or victim and contribute to the identification of the perpetrators [8,9,14].

Determination of the blood group in spots is mainly carried out by the methods of absorption of agglutinins and absorption-elution. In the presence of a sufficient amount of material, the method of absorption of agglutinins in a quantitative modification is used. With a lack of material, then the absorption-elution reaction is used [16,17.18].

Currently, phytohemagglutinins are widely used to determine the blood group according to the AB0 system, both in liquid form and in its traces. These plant substances, extracted from the seeds of some plants, specifically agglutinate the erythrocytes of some systems, including the AB0 system of the human body [1,2,10].

The use of seed extracts as hemagglutinating preparations (lectins) is more economical than the use of expensive immune and isosera. The promise of these agglutinins is determined not only by the economy and technical simplicity of extracting them from plants, but also by the fact that they can have some positive properties that differ from those of serum agglutinins. According to M.I. Potapov [4,12,13], the intensive development of this non-infectious immunology, which successfully competes with classical serum immunology, especially began to develop after the appearance of the works of K.O. Renconen, W. Boyd, E. Shapleigh [11,15].

Purpose of the study. Obtaining phytagglutinins to erythrocyte groups of the AB0 system from extracts of seeds "Saphora Japonica L" and grapes "Nimrang" growing on the territory of the Republic of Uzbekistan.

Based on the foregoing, we, examining the extract of grape seeds “Nimrang” and Saphora Japonica L., growing on the territory of Uzbekistan, established the presence of lectins with the ability to agglutinate human erythrocytes A, B, O [3,7]. They contained polyphytagglutinins that react with two, three agglutinogens (phytagglutinin anti-A + B, anti-A + B + O) and, with appropriate dilutions in saline or in the serum of the fourth blood group of the ABO system, received monophytagglutinins (for example, in seed extract grape "Nimrang" phytagglutinin anti-A, as well as in the seed extract of Saphora Japonica L phytagglutinin anti-B).

Material and methods

60 blood spots were examined (10 of the first group, 22 of the second group, 22 of the third group, and 6 of the fourth group). Isoantigens (isoagglutinogens), isoantibodies (isoagglutinins) of the studied liquid blood before stain preparation were determined by the Schiff test tube method (agglutination reaction), based on the interaction of antigens and antibodies. After examining liquid blood samples, stains were prepared from them on an indifferent carrier object (clean gauze or white cotton satin fabric).

Blood stain antigens were determined by the method of absorption of agglutinins in a quantitative modification given in manuals and textbooks on forensic medicine [5,6]. For control, clean, unstained samples of the carrier object were constantly stained. With clean scissors, 3 weighings of the carrier object weighing 50 mg were cut out, crushed into small pieces, and placed in agglutination tubes with the inscription “f.a-A”, “α” and “f.a-B”, “β”, indicating the name of phytagglutinins and serum alpha, beta (The reaction with alpha and beta sera was used as a comparative control to the extracts "Nimrang" and "Saphora Japonica L"). Then, 4 samples with a blood stain were prepared in the same way and placed in 4 other test tubes, according to the inscriptions. Separately, in test tubes labeled "f.a-A", "f.a-B", 0.3 ml of Nimrang and Saphora Japonica L extracts were added, and in test tubes labeled "α", "β "- 0.3 ml of serum α and β, previously diluted to a titer of 1:32. Test tubes with stains, objects-carriers and left to control the extract and serum were placed in a refrigerator at a temperature of +5-10C for 18-20 hours. After this time, the results of the study were taken into account by testing the absorbed sera and extract with the corresponding standard erythrocytes. To do this, absorbed by the extract "Nimrang" and serum α are titrated with 1% suspension of standard erythrocytes A, extract "Saphora Japonica L" and serum β - 1% suspension of standard erythrocytes B. At the same time, it is revealed whether the serum and extract retained their initial titers or reduced it , and how much. Serums and extracts absorbed by the stain and the carrier object are carefully aspirated from the material, transferred to test tubes with the same inscriptions, and centrifuged for 10-15 minutes until clear. Accounting for absorption results is established by determining the changes that occur in sera and extracts (anti-A phytagglutinin and anti-B phytagglutinin) after absorption. To accomplish this task, on 7 agglutination test tubes with a wax pencil (blue for β and f.a-B, red for α and f.a-A) make inscriptions: H, 2, 4, 8, 16, 32, 64. One Pasteur pipette into all test tubes, except for the first (with the inscription H), add 2 drops of saline. Then the control sera and extracts left the day before in the refrigerator are thoroughly mixed separately. With the same pipette, add two drops of anti-A phytagglutinin control solution (Nimrang extract) into the first two test tubes (labeled H and 2). The

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contents of the tube labeled 2 are thoroughly mixed with the same pipette by suction and blowing, and 2 drops from it are transferred to the tube labeled 4. From the same tube, also after mixing, 2 drops are transferred to the next tube (labeled 8), and from it to test tube labeled 16, and so on until the end of the row. From tube 64 after mixing, 2 drops are removed. Then, approximately the same pipette is selected according to the diameter of the capillary, with the help of which 1% suspension of standard group A erythrocytes is added to all tubes. The tubes are shaken and centrifuged for 4 minutes at 1000-1500 rpm, after which the tubes are shaken vigorously times with equal force. First, the contents of the test tube are viewed with the naked eye. Visible to the eye agglutination in the form of conglomerates of various sizes in the worksheet is indicated by the sign □. The contents of the remaining tubes are microscopically examined. To do this, the contents of the test tubes are turned over onto glass slides, previously marked according to the inscriptions on the test tubes (starting from a dilution of 1:64). The “+” sign indicates the gluing of all erythrocytes into conglomerates of various sizes. The “+” sign corresponds to small conglomerates against the background of a large number of non-adhered erythrocytes. The designation "-/+" corresponds to small agglutinates of 3-5 glued erythrocytes against the background of the majority of non-glued ones. The “-/+” sign denotes single agglutinates of 2-3 glued erythrocytes (on the 2-3 field of view), and the bulk of non-glued erythrocytes. The “–” sign denotes the complete absence of agglutination. The anti-A phytagglutinin (Nimrang extract) retained as a control may remain unchanged and have a titer of 1:32. In the same way, the titer of the control serum α, β and anti-B phytagglutinin (Saphora Japonica L extract) are checked,

After checking the titer of control serum α and phytagglutinin anti-A, the titer of control serum β and phytagglutinin anti-B is checked in the same way with the addition of 1% suspension of standard group B red blood cells. After that, changes in the titer of phytagglutinin anti-A, phytagglutinin anti -B and sera α, β, which are in interaction with the control subjects-carriers and with a blood stain, that is, with the object of study.

Result and discussion

As a result of the absorption of anti-A phytagglutinin under the influence of 22 blood spots of the second group, in 10 of them a decrease in the titer of this agglutinin 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. The serum titer of this phytagglutinin decreased by 1 step under the influence of six carrier objects. Subject-carriers of the remaining 16 blood spots had no effect on the titer of anti-A phytagglutinin. Under the influence of six blood spots of the AB (IV) group, a decrease in the titer of anti-A phytagglutinin by 3-4 steps was observed. Subject carriers of these bloodstains had no effect on the titer of this anti-A phytagglutinin. 14 blood spots of the third group basically (in 11 cases) had no effect on the titer of anti-A phytagglutinin. Only in three cases there was a decrease in the titer of anti-A phytagglutinin by 1 step.

Also, the absorption of anti-B phytagglutinin under the influence of 14 blood spots of the third group, in 8 of them there was a decrease in the titer of this agglutinin by 5-6 steps. In the remaining 6 cases, a decrease in the anti-B phytagglutinin titer by 3-4 steps was observed. The serum titer of this anti-B phytagglutinin decreased by 1 step under the influence of six carrier subjects. Subject-carriers of the remaining 8 blood spots had no effect on the titer of anti-B phytagglutinin. Under the influence of two blood spots of the AB (IV) group, a decrease in the titer of anti-B phytagglutinin by 5-6 steps was observed. Subject carriers of these bloodstains had no effect on the titer of this anti-B phytagglutinin. 22 blood spots of the second group basically (in 18 cases) had no effect on the titer of anti-B phytagglutinin. Only in four cases was a decrease in the anti-B phytagglutinin titer by 1 step observed.

Parallel control studies of alpha serum showed that the titer of this serum, under the influence of 22 blood spots of the second group, decreased by 3-5 steps. The subject - their carriers did not affect the titer of this serum. Under the influence of two blood stains of AB (IV) group, the alpha serum titer decreased by 3-4 steps, and their carriers did not affect the titer of this serum. The titers of phytagglutinins anti-A, anti-B and serum α, β did not change under the influence of 10 blood stains of the first group and subjects carrying these stains.

When studying the results of absorption of agglutinins β under the influence of 14 blood spots of the third group, a decrease in serum titer β by 3-5 steps was observed. Subject-carriers did not affect the serum titer β. Under the influence of 2 blood spots of the fourth group, the serum β titer decreased by 3-4 steps. The titer of serum α and anti-A phytagglutinin did not change under the influence of 14 blood stains of the third group, 10 blood stains of the first group and their carriers.

Conclusions

Thus, the results of a study of 60 blood stains of the first group ($O_{\alpha\beta}$), second (A_{β}), third (B_{α}) and fourth group (AB0) show the possibility of using anti-A phytagglutinin (Nimrang grape seed extract) and anti-B phytagglutinin (seed extract "Saphora Japonica L") for the determination of antigens A and B in traces of blood. Phytagglutinins anti-A, anti-B have a number of advantages compared to alpha and beta isosera, primarily due to their cost-effectiveness. The use of new ingredients in the

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practice of examination enriches the arsenal of methods for examining material evidence.

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