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STUDYING THE EFFECT OF MEDICINAL PLANT PREPARATIONS ON ANTIGEN COMPETITION IN THE IMMUNE RESPONSE

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Abstract: White mice were divided into 8 groups to study the effect of herbal preparations on antigen competition in white mice. When mice are injected with a dominant antigen (EL) 4 days before EB immunization, a dramatic (4.7-fold) suppression of the immune response to EB occurs, meaning that the dominant antigen suppresses the body's immunological response to EB. In mice pretreated with EL, the number of nucleated spleen cells was reduced by 1.3 compared to controls. Gulzor 17-Doctor Ali 6 NSC increases 2.3 times when EL is taken together with "Hepar Neo" balm; Gulzor 17-Doctor Ali 6 "Hepar Neo" balm combined with EB increases the number of AJ by 3.1 times compared to group 2. Similar results were obtained when the number of AJS significantly increased as a result of the introduction of herbal preparations: (EL + Balsam Gulzor) + EB - 1.9 times, EL (EB + Balsam Gulzor) - 2.7 times, (EL + Detoxioma) + EB - 1.7 times and EL+(EB+Detox) - 1.9 times.

Keywords: Herbal medicinal preparations, immune response, antigen, phagocytes, immunization.

INTRODUCTION

Products derived from plants that are used for treating diseases or maintaining health are called herbal products or phytomedicines. When a plant-based product is specifically intended for internal use, it is referred to as a herbal supplement. Many prescription and over-the-counter medications are also derived from plant sources; however, these pharmaceutical products typically contain only purified plant ingredients. In contrast, herbal supplements may include entire plants or specific parts of them. Herbal supplements come in various forms: dried, chopped, powdered, in capsules, or as liquids, and they can be used in different ways depending on the purpose (*Herbal Medicine*).

Guidelines issued by the Ministries of Health in China and South Korea recommend the use of traditional treatment methods at various stages of COVID-19. However, detailed clinical trials are required to determine the effectiveness of these remedies. Cinchona trees have been proven to be effective in the treatment of COVID-19.

Currently, Astragalus mongholicus is among the most widely used medicinal plants. In India, several herbal remedies have been recommended for the prevention of COVID-19, including Tinospora cordifolia, Andrographis paniculata, Cydonia oblonga, Zizyphus jujube, and Cordia myxa. In Morocco, the most commonly used plants for preventive purposes (with usage frequency >40%) include: Allium sativum (80.9%), Olea europaea (72.7%), Allium cepa (66.7%), Zingiber officinale (66%), Thymus maroccanus (59.2%), Eucalyptus globulus (56.5%), Foeniculum vulgare (54.3%), Curcuma xanthorrhiza (50%), Phoenix dactylifera (50%), Rosmarinus officinalis (47.9%), Thymus satureioides (41.9%), Mentha pulegium (41.3%), and Pimpinella anisum (40%).

In recent years, various plant extracts have been studied for their immunomodulatory activity on lymphocytes. Among these extracts, Salvia mirzayanii, derived from several medicinal plants, has shown notable activity. This plant belongs to a large family comprising at least 900 species, found across temperate and subtropical regions of the world (Duke, 1988). The antimicrobial activity of the essential oil of *S. mirzayanii* has been investigated (Sonboli, Babo-khani, & Mehrabian, 2006),

and a novel sesterterpene, salvimirzakolide, has been isolated. *Salvia mirzayanii* is traditionally used in the treatment of infectious and inflammatory diseases.

Medicinal plants have been known to humanity since ancient times. People have used plants not only as food but also as a source of biologically active compounds. There is evidence that medicinal plants were used for therapeutic purposes as early as 5,000 years ago. For a long historical period, medicinal plants served as the sole source of medicines. In the territory of Uzbekistan, about 4,500 species of higher plants grow naturally, of which approximately 1,200 species possess medicinal properties. Currently, 112 species of medicinal plants are officially approved for use in conventional medicine in our Republic, with 80% of them being wild-growing plants.

METHODS

The effects of a plant preparation on the primary immunity of animals were studied in a series of experiments. The femoral muscles of mice were cleaned, epiphyses were cut, and bone marrow was washed from the bone canal of all organs to prepare a cell suspension of the immune system. The number of colony-forming units (JASK) was counted in a Goryaev chamber and recalculated for the entire organ. This method was used to determine the total number of cells in central (thymus, bone marrow) and peripheral (lymph nodes, spleen) immune organs.

The experiments were conducted on white mice weighing 20-22 g, which were immunized with the test drugs instead of EB (Zanjabil Detoxioma, Gulzor Balm, Doctor Ali 1 tea decoction, Gulzor 17 - Doctor Ali 6 “Hepar Neo” balm) at a dose of 0.25 ml/kg. During the experiment, the requirements of the World Society for the Protection of Animals (WSPA) and the European Convention for the Protection of Vertebrate Animals used for Experimental Purposes (Strasbourg, 1986) were observed.

Excel 2013 (Microsoft) applications were used for statistical processing and analysis of the research results, as well as for creating graphs based on the obtained data.

RESULTS

To study the phenomenon of antigen competition in the immune response, mice were first administered horse erythrocytes (ER) as a dominant antigen at a dose of 1x10^9/ml. After 4 days, they were given 2x10^8/ml of sheep erythrocytes (SE) as the immunizing antigen. Four days later, the number of antibody-forming cells (AFC) in the spleen was determined.

To determine the phagocytic activity of leukocytes, the test drugs were administered intravenously to mice once. Four days later, the mice were euthanized, blood samples were collected, and then 50 µl of latex particles (diameter 1–1.5 µm) were added to 50 µl of blood. The mixture was incubated for 30 minutes at 37°C. Smears were prepared, and phagocytosis parameters were determined. Additionally, the leukocyte count was measured.

Three indicators of phagocytosis were identified:

1. Phagocytic Index (PI) – the proportion of phagocytic leukocytes;
2. Phagocytic Number (PN) – the average number of latex particles ingested per leukocyte;
3. Phagocytic Capacity (PC) – the total number of latex particles ingested by leukocytes in 1 µl of blood.

The effect of plant preparations on antigen competition in the immune response.

It is known that the sequential introduction of two antigens causes the first antigen to suppress the immune response to the second antigen. We studied the effect of plant preparations on antigen competition in white mice.

White mice were divided into 8 groups: 1st (control) group received only sheep erythrocytes (SE) at a dose of 2x10^8/ml; 2nd group received horse erythrocytes (ER) at a dose of 2x10^9/ml (dominant antigen) first, and after 4 days were immunized with SE at a dose of 2x10^8/ml (immunizing antigen); 3rd group simultaneously received EL and detoxiome at a dose of 0.25 ml/kg, and after 4 days were immunized with SE; 4th group received EL, and after 4 days were immunized with SE along with detoxiome; 5th group simultaneously received EL and Doctor Ali 1 tea decoction at a dose of 1.5 ml/kg, and after 4 days were immunized with SE; 6th group received EL, and after 4 days were immunized with SE along with Doctor Ali 1 tea decoction; 7th group simultaneously received EL and Gulzor 17 - Doctor Ali 6 “Hepar Neo” balm at a dose of 0.2 ml/kg, and for 4 days were immunized with SE; 8th group received EL, and after 4 days were immunized with SE along with Gulzor 17 - Doctor Ali 6 “Hepar Neo” balm.

Four days after immunization, antibody generation in the spleen was determined.

The effect of herbal preparations on the competition of antigens (horse and sheep erythrocytes) in mice (M±m)

Table 1

Group (n=8)	Days of drug administration	Number of CFU x10⁶/ml	IP	Number of AFC per spleen	IP
Control + (EB)	-	-	206.0 ± 10.0	-	3822.0 ± 31.0
EL + EB	-	-	158.0 ± 9.0 a	-1.3	815.0 ± 10.0 a
(EL + Detoxioma) + EB	+	-	267.0 ± 14.0 b	+1.7	3214.0 ± 32.0 b
EL + (EB + Detoxioma)	-	+	308.0 ± 15.0 b	+1.9	4234.0 ± 39.0 b
(EL + Doctor Ali 1 tea infusion) + EB	+	-	195.0 ± 11.0 b	+1.2	1981.0 ± 9.0 b
EL + (EB + Doctor Ali 1 tea infusion)	-	+	264.0 ± 14.0 b	+1.7	2784.0 ± 21.0 b
(EL + Gulzor balm) + EB	+	-	301.0 ± 19.0 b	+1.9	3147.0 ± 22.0 b
EL + (EB + Gulzor balm)	-	+	428.0 ± 25.0 b	+2.7	4097.0 ± 29.0 b
(EL + Gulzor 17 - Doctor Ali 6 "Hepar Neo" balm) + EB	+	-	367.0 ± 17.0 b	+2.3	3618.0 ± 27.0 b
EL + (EB + Gulzor 17 - Doctor Ali 6 "Hepar Neo" balm)	-	+	489.0 ± 27.0 b	+3.1	4856.0 ± 38.0 b

Note:

EL - horse erythrocytes, IP - index ratio, (-) - compared to 1st group, (+) - compared to 2nd group, a - significant difference from control, b - significant difference from 2nd group, (n=8) - number of animals in the group.

The results showed that if the dominant antigen (EL) is administered to mice 4 days before EB immunization, there is a sharp suppression (4.7-fold) of the immune response to EB, meaning that the dominant antigen suppresses the body's immunological response to EB.

Administering the Gulzor 17-Doctor Ali 6 “Hepar Neo” balm to mice along with the dominant EL antigen completely neutralizes the ability of the latter to suppress the immune response to EB. In group 9 mice, the level of AFC is 4.4 times higher than in groups 2 and the control, reaching comparable levels.

Co-administration of Gulzor balm and detoxioma with the dominant antigen significantly increased the AFC count by 3.9 times. In the group treated with Doctor Ali tea infusion 1, the AFC level increased by 2.4 times.

A similar pattern was observed when herbal preparations were used together with EB. The AFC counts in the spleens of mice from groups 3, 8, and 10 were 5.2, 5.0, and 6.0 times higher, respectively, compared to animals previously given EL.

DISCUSSION

It should be emphasized that the second variant of administering herbal preparations (with either EL or EB) proved to be more effective.

When herbal preparations were administered with both the dominant antigen and the immunizing antigen, they clearly abolished the antigen competition in the immune response.

In mice pre-treated with EL, the number of nucleated spleen cells decreased by 1.3 times compared to the control. When EL was administered together with Gulzor 17-Doctor Ali 6 "Hepar Neo" balm, the number of nucleated spleen cells increased by 2.3 times; co-administration of Gulzor 17-Doctor Ali 6 "Hepar Neo" balm with EB increased the number of nucleated spleen cells by 3.1 times compared to group 2.

Similar results were obtained when the number of nucleated spleen cells significantly increased after the introduction of herbal preparations:

- (EL + Gulzor Balm) + EB increased 1.9 times,
- EL (EB + Gulzor Balm) increased 2.7 times,
- (EL + Detoxioma) + EB increased 1.7 times,
- EL + (EB + Detoxioma) increased 1.9 times.

Thus, the ability of herbal preparations to abolish the phenomenon of antigen competition in mice was clearly demonstrated.

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