EFFECT OF STEVIA REBAUDIANA ON MORPHO-FUNCTIONAL INDICATORS OF PERIPHERAL BLOOD

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Rich in natural antioxidants, Stevia rebaudiana has immunoregulatory, anti-stress, anti-inflammatory, antiviral, antihistaminic properties, increases the body's nonspecific defense, bioenergetic level, and improves tissue respiration. We have studied the long-term effect of S. rebaudiana on morphofunctional parameters of peripheral blood.

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Introduction.

The impaired functions of the body are more efficiently regulated and restored by biochemical remedies. From this point of view, herbs are an indispensable remedy for the treatment or relief of disease phenomena occurring in a living organism. They are relatively safe and can be used in any conditions [1, 2].

Herbs containing glycosides and flavonoids are distinguished by the content of physiologically active compounds and therapeutic properties. One such species is S. rebaudiana, commonly known as sweetleaf. Sweetleaf, rich in natural antioxidants, has immunoregulatory, anti-stress, anti-inflammatory, antiviral, antihistaminic properties, increases the body's nonspecific resistance, the level of bioenergetics and provides tissue respiration [3–5].

The structure of glycosides in sweetleaf is similar to that of human hormones, so it can protect the body from stress and harmful effects on the environment, can help overcome hormonal disorders [6, 7]. Stevioside in sweetleaf plays an important role in the synthesis of hemoglobin proteins, which is promoted by iron, lysine amino acids, copper, flavonoids, vitamins C, B₂, E. Therefore, sweetleaf is useful at reduced physical activity and mobility. In addition, sweetleaf contains antioxidants, minerals, amino acids, tannins.

In the literature we studied, we did not find dynamic studies of the long-term effect of sweetleaf on the parameters of peripheral blood, which prompted us to conduct this study.

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Materials and Methods.

The experiments were carried out on male rabbits weighing 2.5–3 kg under the same feeding conditions. For 30 days, the animals received crushed dry leaves of sweetleaf with food at the rate of 0.5 g per 1 kg of body weight.

Under normal conditions and on the 5th, 10th, 15th, 20th, 25th, 25th and 30th days of feeding with sweetleaf, the following morphofunctional parameters of the peripheral blood were studied: the red and white blood cell counts, hemoglobin content, color index (Farben index), relative and absolute number of reticulocytes, maturation rate of reticulocytes per hour, differential white blood cell count.

The erythrocyte and leukocyte counts were determined by Goryaev’s counting-chamber device (hemocytometer), the hemoglobin content was determined by Sahli’s hemoglobinometer. To extract the color index, the amount of hemoglobin in the blood (in Sahli’s units) was divided by doubling the first two digits of the red blood cell count. The relative percentage of reticulocytes was determined by the Egorov method, and to determine the absolute number, we multiplied the number of erythrocytes in the blood of the given day by the relative percentage of reticulocytes of the same day and divided by 100.

To determine the maturation rate of reticulocytes per hour, we took blood 4 times with a Panchenkov’s pipette, poured it into a test tube, added 1 drop of heparin to prevent coagulation, and placed it in a thermostat for 4 h at 37°C. Then we took blood with a Sahli’s hemoglobin pipette, put it in a small test tube, added the same amount of Romanowsky stain to it. Two hours later, a smear was prepared, where 1000 erythrocytes were counted, among which the reticulocytes were distinguished. To determine the maturation rate of reticulocytes per hour, we subtracted the number of reticulocytes counted after incubation from that counted before incubation, and divided the resulting number by the incubation time.

To determine the differential white blood cell count, a blood smear was prepared, which was fixed with ethyl alcohol for 10 minutes, then stained with azure B–eosin Y (Romanowsky–Giemsa staining). In the smear using the immersion system, 200 cells were counted (according to Schilling). To determine the absolute number of different leukocytes, we multiplied the relative percentage of these types by the leukocyte count in the blood of the same day and divided by 100.

Results and Discussion.

Studies have shown that using the sweetleaf when feeding rabbits for 30 days stimulated hematopoiesis.

On the 5th day of the study, a moderate normochromic increase in the erythrocyte count and of the hemoglobin content was observed, when the color index did not change significantly (table 1). During this period, reticulocytosis was observed. The relative number of reticulocytes was 122.0% relative to the initial amount, and the absolute number was 130.0%.

By the 10th day of the study, a normochromic increase in the number of erythrocytes and hemoglobin content continued. The relative number of reticulocytes was 138.8%, and the absolute – 163.4%. The maturation rate of reticulocytes reached 166% relative to the initial one. These changes indicated a moderate activation of erythropoiesis.
By the 15th day of the study, a hyperchromic change in the number of erythrocyte and hemoglobin content was observed. Compared to the 10th day, the red blood cell count decreased by 4% and the hemoglobin content increased by 5%, resulting in the increase of the color index from 0.80 to 0.85. High levels of relative and absolute reticulocyte counts were maintained (161%, $p<0.001$; 179%, $p<0.001$, respectively), with a predominance of the first and second groups of reticulocytes not normally found in peripheral blood. The latter was a manifestation of increased erythropoiesis.

On the 20th day of the study, a moderate normochromic decrease in the erythrocyte count and hemoglobin content was observed, resulting in the color index to recover the initial level (0.80). The relative and absolute count of reticulocytes reached a maximum (161%, $p<0.001$; 178.8%, $p<0.001$, respectively).

On the 25th day of the study, the previous high level of reticulocytosis and a high rate of maturation of reticulocytes had a positive effect on the erythrocytes count and the hemoglobin content. The number of erythrocytes and the content of hemoglobin increased by 17% compared with the norm.

By the 30th day, the studied hematological parameters of peripheral red blood remained at a moderately high level.

Such dynamics of peripheral red blood cell count, similar to that observed in animals receiving sweetleaf with food for 30 days, was probably due to the presence of biologically active substances in sweetleaf. According to the literature, stevioside plays an important role in the synthesis of hemoglobin proteins, which is promoted by iron, lysine amino acids, copper, flavonoids, vitamins C and B2, and organic acid converts inactive folic acid into active one [3, 8].

Table 2

The effect of sweetleaf feeding on the hematological parameters of peripheral red blood cell

| Hematological parameter | Initial data | Research days |
|-------------------------|-------------|---------------|
|                         |             | 5 10 15 20 25 30 |
| Erythrocytes (10^9/L)   | 4700±155    | 5025±163 5560±173 5402±168 5226±161 5540±171 5491±169 |
|                         |             | p<0.001 p<0.02 p<0.05 p<0.01 p<0.001 |
| Hemoglobin (g/dL)      | 12.6±0.22   | 13.8±0.24 14.8±0.25 15.4±0.25 14±0.23 14.8±0.23 14±0.22 |
|                         |             | p<0.01 p<0.01 p<0.02 p<0.01 p<0.02 |
| Colour index           | 0.80        | 0.82 0.80 0.85 0.80 0.76 0.76 |
| Reticulocytes percentage (%) | 18±0.38 | 22±0.45 25±0.26 28±0.48 29±0.51 21±0.42 21±0.41 |
|                         |             | p<0.02 p<0.001 p<0.001 p<0.001 p<0.02 p<0.02 |
| Reticulocytes (10^9/L) | 846±26.4    | 1105±34 1382±35 1512±36 1515±36 1163±34 1153±34 |
|                         |             | p<0.01 p<0.001 p<0.001 p<0.001 p<0.01 p<0.01 |
| Maturation rate of reticulocytes per hour | 1.5 | 1.25 2.5 2.4 2.5 2.0 1.5 |
Table 2 shows the dynamics of changes in the peripheral white blood cell count of animals that received sweetleaf for 30 days. According to the table, 30 days of feeding with sweetleaf had a stimulating effect on leukopoiesis.

On the 5th day of the study, there was a moderate increase in the leukocyte count (117% relative to the initial amount, p<0.05) due to an increase in the number of neutrophils and lymphocytes. The band neutrophil count increased to 120% (p<0.02), the segmented neutrophil count – up to 113% (p<0.05), and the number of lymphocytes – up to 120% (p<0.05).

On the 10th day, the increase in the total number of leukocytes continued (132%, p<0.001). In the leucocyte count, neutrophilia with left nuclear shift, eosinophilia, lymphocytosis, monocytosis were observed.

On the 15th day of the study, the total number of leukocytes did not change significantly compared to the previous day. In the leucocytes count, neutrophilia with degenerative nuclear shift to the left was observed, the number of band neutrophils doubled (202%, p<0.001). Myelocytes and metamyelocytes were present in the blood smear, which is the result of increased functional activity of the bone marrow. In the cytoplasm of neutrophilic cells, toxic granulation was observed, which, according to Freifeld, is not the result of transformation processes in the cell, but a manifestation of the reactivity of these cells. During this period, a high level of lymphocytes remained (148%, p<0.001). The eosinophil count reached its maximum (168%, p<0.001).

### Table 2

| White blood cells          | Initial data | 5   | 10  | 15  | 20  | 25  | 30  |
|----------------------------|--------------|-----|-----|-----|-----|-----|-----|
| Leukocytes (10^6/L)        | 8000±220     | 9400±255 p<0.05 | 10600±395 p<0.001 | 10800±389 p<0.001 | 11500±396 p<0.001 | 1000±281 p<0.001 | 9600±245 p<0.01 |
| Band neutrophils (10^6/L)  | 40±3         | 48±3 p<0.02    | 53±4 p<0.001       | 81±5 p<0.001       | 86±4 p<0.001       | 50±3 p<0.02       | 48±3 p<0.05     |
| Segmented neutrophils (10^6/L) | 2960±118 | 3357±111 p<0.05 | 3486±115 p<0.01 | 3435±135 p<0.01 | 4140±138 p<0.001 | 3600±135 p<0.01 | 3600±13 p<0.001 |
| Eosinophils (10^6/L)       | 160±11       | 211±13 p<0.001 | 265±16 p<0.001    | 270±16 p<0.001    | 230±12 p<0.001    | 200±11 p<0.01    | 192±12 p<0.01  |
| Basophils (10^6/L)         | 40±3         | 48±3 p<0.02    | 53±3 p<0.001      | 54±3 p<0.001      | 58±4 p<0.001      | 50±3 p<0.02      | 48±3 p<0.02    |
| Monocytes (10^6/L)         | 480±12       | 519±13         | 583±17 p<0.001    | 560±16 p<0.001    | 546±12 p<0.02     | 600±18 p<0.001   | 576±13 p<0.001 |
| Lymphocytes (10^6/L)       | 4320±155     | 5217±175 p<0.001 | 6160±180 p<0.001 | 6400±196 p<0.001 | 6440±173 p<0.001 | 5500±161 p<0.001 | 5136±162 p<0.001 |
On the 20th day of feeding with sweetleaf, the total leukocyte count remained at a high level (143.7%, p<0.001), the lymphocyte and band and segmented neutrophil counts continued to increase (149%, p<0.001; 215%, p<0.001; 139%, p<0.001, respectively). Plasma cells and reticular stromal cells were found in blood smears.

Stabilization of leukocytes count at a moderately high level was observed on the 25th and 30th day (125%, p<0.001; 120%, p<0.001, respectively). In the differential white blood cell count, a high level of all morphological types of leukocytes was maintained.

**Conclusion.**

The data obtained suggest that changes in the activity of the sympathoadrenal system, increase in non-specific resistance, activation of endogenous immunoregulators may play a role in the mechanism of physiological action of biologically active substances contained in *S. rebaudiana*, which contribute to the mobilization of compensatory mechanisms, affect the metabolism of bone marrow stem cells and ensure the balance of sympathetic and parasympathetic mechanisms of hematopoiesis regulation.

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EFFECT OF S. REBAUDIANA ON MORPHO-FUNCTIONAL INDICATORS OF BLOOD

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ВЛИЯНИЕ СТЕВИИ МЕДОВОЙ НА МОРФО-ФУНКЦИОНАЛЬНЫЕ ПОКАЗАТЕЛИ ПЕРИФЕРИЙНОЙ КРОВИ

Богатая природными антиоксидантами, стевия медовая обладает иммунорегуляторными, антистрессовыми, противовоспалительными, противовирусными, антигистаминными свойствами, повышает неспецифическую защиту организма, биоэнергетический уровень и улучшает тканевое дыхание. Мы изучили долгосрочное влияние стевии медовой на морфофункциональные параметры периферической крови.