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# Effects of Prophylactic Use of a Decoction of Blueberry Leaves in Modeling the Lesion of the Gastrointestinal Tract by Non-Steroidal Anti-Inflammatory Drugs

## Efekty profilaktycznego stosowania wywaru z liści borówki czarnej w modelowaniu uszkodzenia przewodu pokarmowego niesteroidowymi lekami przeciwzapalnymi

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### SUMMARY

**Introduction:** Today there is a continuing search for an effective, affordable remedy that can be used to treat and prevent diseases of periodontal, stomach and duodenum during the use non-steroidal anti-inflammatory drugs (NSAIDs).

**Aim:** To study the protective effect of phytopreparation (decoction of blueberry leaves) on an experimental model of gastric ulcer and periodontitis.

**Material and Methods:** The study was performed on 30 rats, which were evenly divided into three groups: 1 – control animals, 2 – animals that were modeled with non-steroid gastric ulcer and periodontitis (7 mg/kg diclofenacum natrium intragastrically for 5 days) and 3 – group in the background experimental pathology was introduced decoction of blueberry leaves 1:10 2 ml intragastrically (30 minutes after the introduction of diclofenacum natrium).

**Results:** Histological examination of the mucous membrane of rats, which used as a preventive agent decoction of blueberry leaves, showed that it is characterized only by single erosion, no fibrinosis and hemorrhagic plaque, lympho-macrophage infiltration.

**Conclusions:** Simultaneous use of a decoction of blueberry leaves can be used as a preventive and curative agent to prevent the development of erosions and ulcers of the mucous membrane of the digestive system.

**Key words:** NSAIDs, prevention the development of erosions and ulcers, decoction of blueberries leaves

### STRESZCZENIE

**Wprowadzenie:** Obecnie trwają poszukiwania skutecznego, niedrogiego środka, który można stosować do leczenia i zapobiegania chorobom przyzębia, żołądka i dwunastnicy podczas stosowania niesteroidowych leków przeciwzapalnych (NLPZ).

**Cel:** Zbadanie ochronnego efektu fitopreparacji (wywar z liści borówki) na eksperymentalnym modelu wrzodu żołądka i zapalenia przyzębia.

**Materiał i metody:** Badanie przeprowadzono na 30 szczurach, które podzielono równomiernie na trzy grupy: 1 – zwierzęta kontrolne, 2 – zwierzęta, które zostały wymodelowane z niesteroidowym wrzodem żołądka i zapaleniem przyzębia (7 mg/kg diclofenacum natrium dożołądkowo przez 5 dni) i 3 – grupa z wprowadzoną eksperymentalną patologią oraz leczeniem wywarem z liści borówki 1:10 2 ml dożołądkowo (30 minut po wprowadzeniu ortofenu).

**Wyniki:** Badanie histologiczne błony śluzowej szczurów, którym zastosowano jako środek zapobiegawczy wywar z liści borówki, wykazało że ona charakteryzuje się tylko pojedynczymi erozjami, brakiem włóknienia i płytki krwotocznej, naciekaniami limfocytów i makrofagów.

**Wnioski:** Jednoczesne stosowanie NLPZ i wywaru z liści borówki może być stosowane jako środek zapobiegawczy i leczniczy erozji i wrzodów błony śluzowej układu pokarmowego.

**Słowa kluczowe:** NLPZ, zapobieganie rozwojowi erozji i wrzodów, wywar z liści borówki

## INTRODUCTION

In the structure of the prevalence of diseases of the digestive system, peptic ulcer of the stomach and duodenum accounts for 12.83%. According to the Ministry of Health of Ukraine, the incidence of ulcer in the stomach and duodenum in Ukraine in 2010 amounted to 127.3 people per 100 thousand population (absolute values – 58293.0), prevalence – 2299.4 per 100 thousand population (absolute value – 1052741.0). The ulcer is a damage to the epithelium that spreads to the muscular plate of the mucous membrane. Peptic ulcer can lead to the development of a number of complications, including urgent, such as acute gastrointestinal bleeding or perforations. Among the factors that provoke peptic ulcers, is uncontrolled use of nonsteroidal anti-inflammatory and antirheumatic drugs (NSAID) [1, 2].

Every day in the world more than 30 million people use NSAIDs, during the year the number of NSAIDs is more than 300 million people, while only every third patient takes NSAIDs as prescribed by a doctor. At the end of the twentieth century, and at the beginning of the current century there is a tendency to increase NSAIDs consumption by 2-3 times every 10 years [3, 4]. The increase in the proportion of elderly and old people in most countries of the world and, consequently, the increase in the prevalence of diseases of the musculoskeletal system with increasing age of patients contribute to the widespread use of NSAIDs.

However, when using NSAIDs, which are among the most common drugs, drug safety and comorbidity are closely intertwined.

From complications caused by NSAIDs, up to 2 thousand patients die annually in the UK, in the United States 16.5 thousand deaths per year and 107 thousand hospitalizations are associated with the use of NSAIDs [3-5]. Adverse side effects are characteristic of almost all members of the NSAIDs group (with different frequency), regardless of their chemical structure, dosage form, and route of administration.

This problem is relevant not only in the medical, but also in the socio-economic aspect, significantly increasing the number of hospitalizations and deaths, as evidenced by the data of numerous epidemiological studies [6]. Side effects of NSAIDs are class-specific, characteristic of all members of this group of drugs, and are determined by their main pharmacological action – cyclo-oxygenase enzyme blockade.

NSAID interrupts the cyclooxygenase pathway of metabolism of arachidonic acid, thereby violating the synthesis of prostaglandin in the mucous membrane of the stomach and the mucous membrane of the duodenum by suppressing prostaglandin synthase, thereby exhausting their tissues and causing iatrogenic “prostaglandin deficiency” [7].

At the same time there is a relaxation of the mucosal-bicarbonate barrier, decreased cellular proliferation and physiological regeneration of the epithelium, deterioration of the microcirculation and regional blood circulation. Therefore, at the same time, all levels of protection of mucous membrane (preepithelial, epithelial) are struck

post- epithelial defense), which creates the prerequisites for damage to stomach and duodenum [8, 9].

The second mechanism for the emergence of NSAID-induced gastropathy and periodontitis is the activation of pericidal lipid oxidation, which results in free radicals accumulation in tissues, among which hydrogen peroxide and hydroxyl radical are most dangerous for the mucous membrane [10].

The third pathogenetic mechanism of the mucous membrane cells damage is the ability of the NSAIDs to suppress the synthesis of nitric oxide (NO), which physiologically supports regional blood flow, inhibits adhesion and activation of neutrophils, “catches” free radicals, reduces the increased tone of the smooth muscle (including the gastrointestinal tract), characterized by cytoprotective and anti-inflammatory (inhibits the synthesis and expression of cytokines) actions. That is why today much attention is devoted to the creation of new NSAIDs that release NO (NO-aspirin, NO-naproxen) [11].

Despite the century-long history of the use of NSAIDs in clinical practice, the issue of eliminating side effects remains unresolved, among them the greatest proportion is ulcerogenic [12].

Common pathogenetic mechanisms that lead to the simultaneous damage of the digestive system in pathological conditions have been disclosed today [13], that's why there is a continuing search for an effective, affordable remedy that can be used to treat and prevent diseases of periodontal, stomach and duodenum during NSAIDs treatment.

Common blueberry (*Vaccinium myrtillus* L.) is a deciduous shrub with angularly ribbed branches. Honey plant, valuable food and medicinal plant. It has anti-inflammatory, antiseptic, wound healing and diuretic effects. The use of blueberries in medicine as an astringent for various gastrointestinal diseases is mainly due to the high content of tannins (galocatechin, epicatechin, epigallocatechin (up to 12%). Blueberry shoots and leaves contain more than 14 anthocyanins and their derivatives (from 300 to 700 mg%): 3-O-arabinosides, 3-O-glucosides, 3-O-galactosides, cyanidin, delphinidin, petunidin, peonidin, malvidin, idanine, myrtillin, peonidin. Also found other flavonoids (rutin, hyperin, hyperoside, quercetin, avicularin, quercitrin, isoquercitrin, kaempferol, etc.), sugar (glucose, fructose, sucrose) (5-20%); organic acids (citric, oxalic, malic, succinic, quinic, lactic (5-7%), vitamins: ascorbic acid (up to 250 mg%), B2 (up to 0.04 mg%), carotene (up to 1.6 mg%), carotenoids (lutein, zeaxanthin), phenols and their derivatives (hydroquinone, asperuloside, monotropeoside), phenol carboxylic acids (caffeic, chlorogenic, gallic, cinnamic, ferulic, etc.), steroids ( $\beta$ -sitosterol, campesterol, etc.) iridoids (monotropeoside, asperuloside), fatty acids, pectin substances, macro and microelements (accumulates manganese, copper, chromium, iron). There are also many condensed tannins (7-20%), glycosides neomyrtillin (up to 2%) and myrtillin (up to 1%) are contained, there are other phenolic compounds – arbutin, methylarbutin (1-2%), triterpene saponins (ursular and oleanic acids,  $\beta$ -amyirin), alkaloids (myrtine), aliphatic

hydrocarbons. It has been established that the hypoglycemic (sugar-reducing) effect of blueberry shoots is associated with the hexatomic cyclic alcohol inositol, which is the aglycone of neomyrtillin. Due to their insulin-like action, blueberry shoots have found application in antidiabetic preparations. The leaves and fruits of blueberries are used as a vitamin-containing remedy for hypo- and avitaminosis (scurvy), as well as externally for stomatitis, gingivitis as an astringent and antiseptic. The beneficial properties of common blueberries are used in folk medicine for indigestion, pain in the stomach and intestines, for diarrhea, for hemorrhoidal bleeding in the form of an enema, as a diuretic for cystitis and kidney stones. Fresh blueberries are taken for gout, rheumatism, constipation, anemia and other types of diseases, with impaired metabolism. The leaves are used for urethritis, pyelitis, liver diseases. A decoction of fruits or an infusion of leaves in the form of lotions for skin diseases, long-term non-healing wounds and ulcers is used externally [14].

That is why it was interesting to use a decoction of blueberry leaves as a preventive and therapeutic agent at experimental pathology.

## AIM

To study the protective effect of phytopreparation (decoction of blueberry leaves) on an experimental model of gastric ulcer and periodontitis.

## MATERIALS AND METHODS

Experimental studies were performed at thirty white male outbred laboratory rats kept on the standard diet of the vivarium of Vinnytsia National Medical University. The keeping and manipulation of animals was carried out in accordance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress at Bioethics (Kyiv, 2001), and was guided by the recommendations of the European Convention for the Protection of Vertebrate Animals for Experimental and Other Scientific Purposes (Strasbourg, 1986) and the provisions of the "Rules for the clinical safety assessment of pharmacological agents (GLP)". The experiment was authorized by the Commission on Bioethics of Vinnytsia National Medical University (Protocol №1 of January 15, 2020), which found that the studies meet ethical and moral requirements under the order of the Ministry of Health of Ukraine №3447-IY "About protection of animals from abuse" of February 21, 2006. The research was performed according to the methodical recommendations "Observance of ethical and legal norms and requirements when performing scientific morphological research".

Among the experimental animals there were thirty white laboratory adult male rats, aged 3-4 months, weighing 160-180 grams, which were evenly divided into three groups: 1- control animals, 2- animals that were modeled with non-steroid gastric ulcer and periodontitis (7 mg/kg diclofenacum natrium intragastrically for 5 days) and 3 - group in the background experimental pathology was introduced decoction of blueberry leaves 1:10 2 ml intragastrically (30 minutes after the introduction of diclofenacum natrium we brewed 10 ml

of boiling water 1 g of dried blueberry leaves, cooled, took 2 ml and injected intragastrically using a special cannula). At the end of the experiment, the mucous membrane of the stomach and oral cavity of rats was studied by conventional histological method, staining of histological preparations was made with hematoxylin-eosin.

## RESULTS

The gastric mucosa of animals with experimental pathology was littered with erosion and ulcers. Erosions were flat superficial defects of the gastric mucosa with a diameter of 1-2 mm, covered with fibrinous or hemorrhagic plaque and often surrounded by a corolla of hyperemia. In some cases, they were found on visually unchanged gastric mucosa. They were more often localized in the fundus department of the stomach, but they also met in the cardiac department. In addition to erosions, we observed subepithelial hemorrhages of the petechial type, but without disturbance of the integrity of the gastric mucosa (hemorrhagic erosion). Morphologically, they were characterized by changes in the vessels of the microcirculatory bed, lymphoid-macrophage infiltration of the stroma, and focal hemorrhages leading to acute erosive damage to the epithelium. Erosions were combined with dystrophic changes of the gastric glands, inflammatory infiltration by neutrophils and lymphoplasmic elements, edema and hemorrhages into the gastric mucosa. We studied gastric ulcers macro- and microscopically (Figure 1).

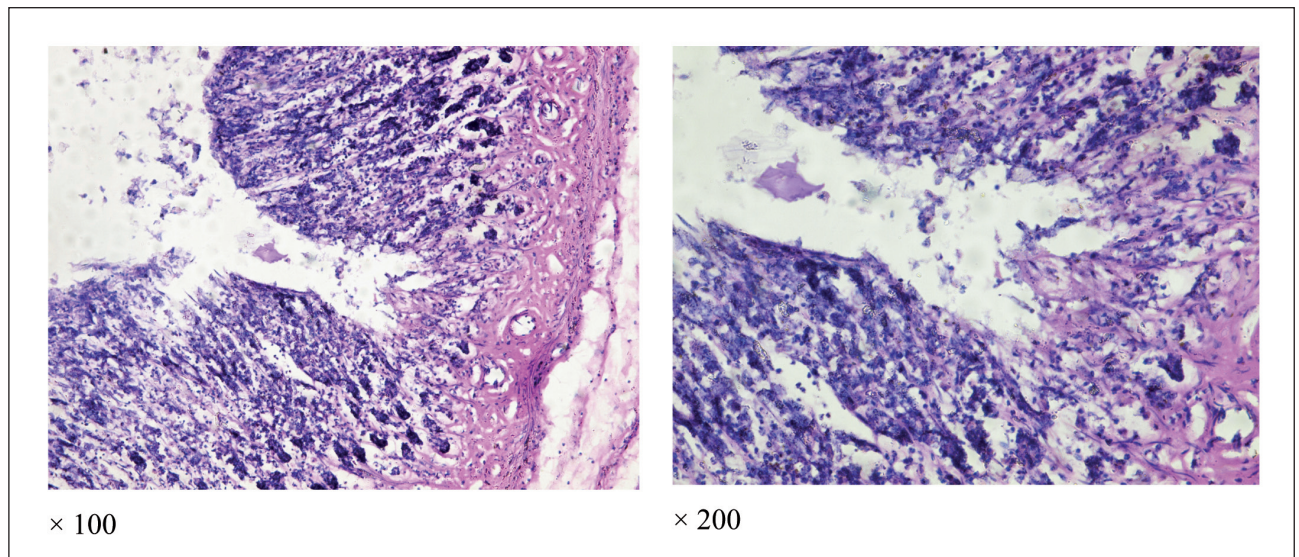
We diagnosed peptic ulcer of the gastric mucosa in the case when microscopically confirmed the defect of the mucous membrane, which penetrated the mucous membrane.

Histological examination of the mucous membrane of rats, which used as a preventive agent decoction of blueberry leaves, showed that it is characterized only by single erosion, no fibrinosis and hemorrhagic plaque, lympho-macrophage infiltration (Figure 2).

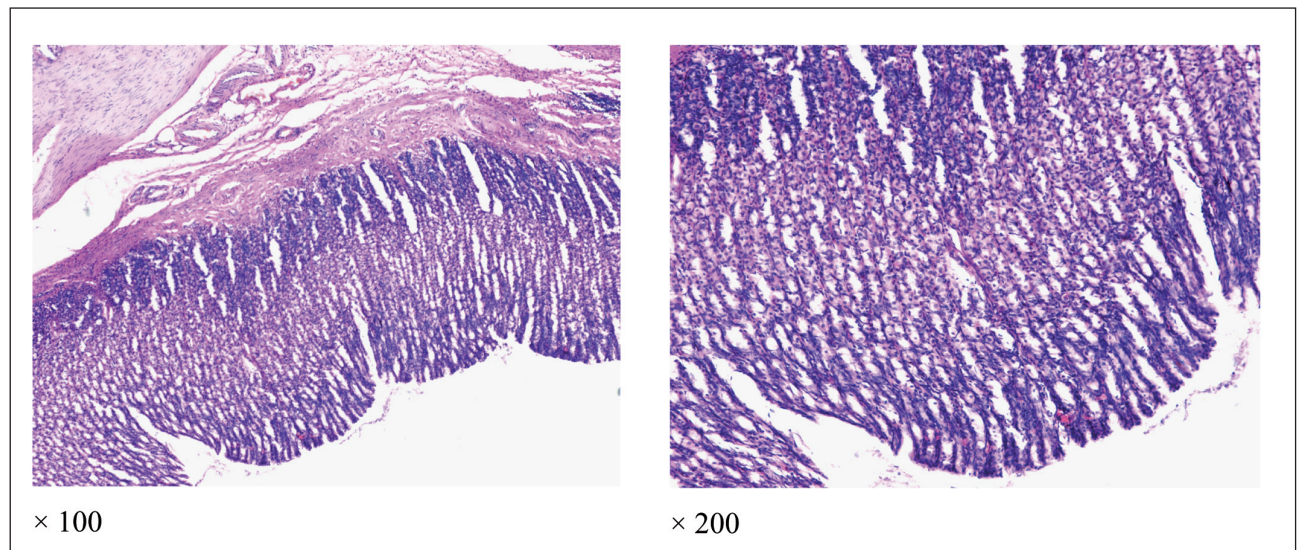
Histological examination of the gum tissue of a group of animals injected with NSAIDs revealed inflammatory changes in the gum tissue almost to the depth of bone tissue.

The area of damage was quite common, without clear boundaries, with the development of necrosis in the center with perifocal reactive changes in the form of moderate inflammation and minor microcirculation disorders. The thickness of the necrosis zone was 0.1 mm, the zone of reactive changes - up to 2 mm. The fibrous tissue of the gums consisted of bundles of collagen and elastic fibers directed in different directions. Disorders of microcirculation were moderate. The vessels in the affected area were slightly dilated, with slight dystrophic changes in the endothelium, moderately full-blooded, with marginal leukocytes in some of them. Polymorphonuclear neutrophils predominated in the inflammatory infiltrate, and single mononuclear cells - lymphocytes and monocytic cells - were also determined.

There was a significant accumulation of edematous fluid with defibering of collagen and elastic fibers of fibrous tissue. Mononuclear cells such as blood monocytes, lymphocytes, and single segmental leukocytes were also detected in the edematous fluid. There was a significant development of granulation tissue



**Figure 1.** The gastric mucosa of animals with experimental pathology

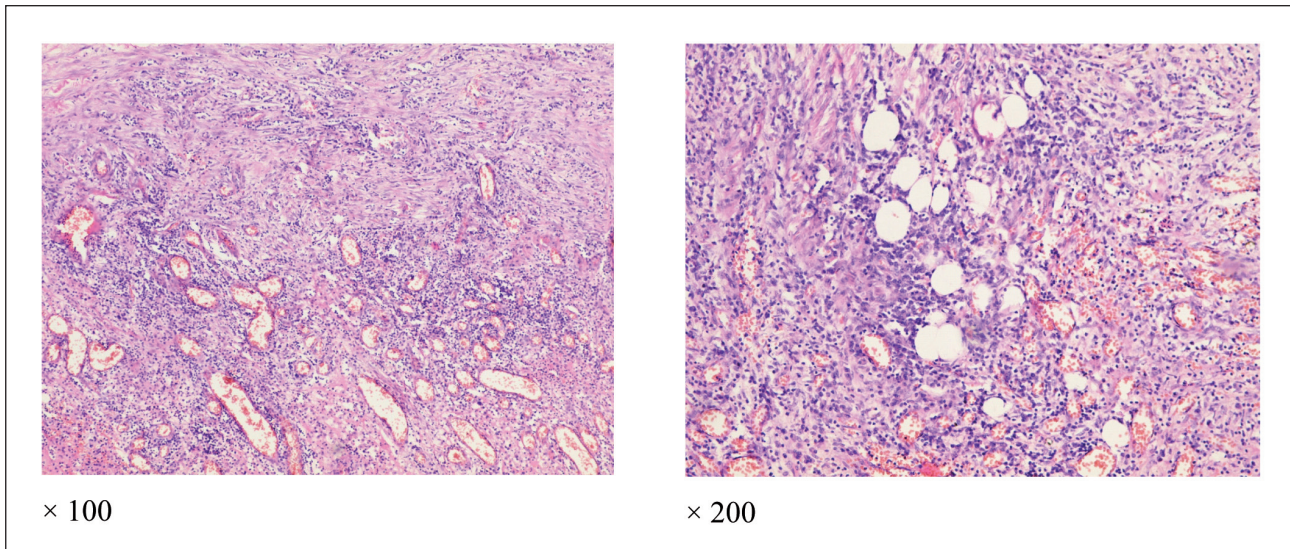


**Figure 2.** The gastric mucosa of animals with experimental pathology of rats, which used as a preventive agent decoction of blueberry leaves

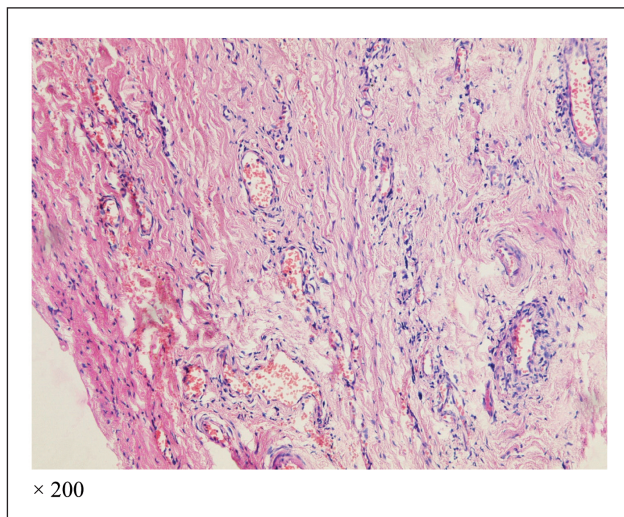
at the site of damaged tissues. Its main components were vertical vascular loops and leukocyte-necrotic zone. The formation of new small vessels with the proliferation of capillary endothelial cells, so-called angioblasts, was observed, which first formed cellular cords, and then from them formed vascular tubes, to which blood flowed. Uniform elements of blood and edematous fluid came out of the young newly formed vessels into the surrounding tissues. Among the inflammatory cells, young forms of fibroblasts were identified. The cellular composition of the infiltrate was represented by segmental neutrophils, among which a small number of lymphocytes and cells of the monocyte series (macrophages, giant multinucleated foreign body cells) were determined (Figure 3).

Concomitant use of NSAIDs and blueberry leaves decoction also determined the area of tissue inflammation to the depth

of the entire gum tissue, but it was much smaller than in rats in the treatment of NSAIDs without the use of blueberry leaf decoction. Proliferation processes were also more pronounced, namely the development of granulation tissue. The area of necrotized tissues was almost undetectable. The newly formed young granulation tissue contained almost no collagen fibers, was infiltrated with a small number of segmental leukocytes and a moderate number of lymphohistiocytic elements, contained a significant number of young fibroblasts. It also showed the formation of a large number of new small vessels with the proliferation of capillary endothelial cells, so-called angioblasts, which first formed cellular cords, and then formed from them vascular tubes, which received blood. In the surrounding tissues from the young newly formed vessels came out shaped elements of blood and edematous



**Figure 3.** Histological examination of the gum tissue of animals with experimental pathology



**Figure 4.** Histological examination of the gum tissue of animals with experimental pathology, which used as a preventive agent decoction of blueberry leaves

fluid in small quantities. The vascular reaction was expressed in the form of moderate plethora, edema of fibrous tissue in the area of inflammation. Single bundles of collagen fibers of dense fibrous decorated connective tissue with insignificant dystrophic changes were also determined

### CONCLUSIONS

Simultaneous use of a decoction of blueberry leaves can be used as a preventive and curative agent to prevent the development of tissue damage of the digestive system.

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*Informacja prasowa*

## TERAPIA MITOCHONDRIALNA

Mitochondria znajdują się w komórce każdej naszej tkanki. Są to nasze “wewnętrzne elektrownie” zaopatrujące organizm w niezbędną do funkcjonowania energię. Dobra forma mitochondriów sprzyja zdrowiu i dobremu samopoczuciu. Osłabione mitochondria mogą być przyczyną wielu schorzeń i chorób przewlekłych.

Terapia mitochondrialna (terapia IHHT) to trening komórek organizmu poprzez podawanie pacjentowi naprzemiennie powietrza z niskim i wysokim stężeniem tlenu.

Terapia jest dobrym wyborem przy ciężkich przewlekłych chorobach.

Dlaczego mitochondria są tak ważne? Mitochondria to centra energetyczne naszego organizmu.

Znajdują się w każdej komórce i to właśnie one zaopatrują organizm w energię niezbędną do życia i funkcjonowania każdego organu. Pełnią również szereg innych istotnych funkcji, chociażby odtruwają nasz organizm.

Komórki mają zdolność do regeneracji i są w stanie odtwarzać niewielkie ubytki mitochondriów.

Jednak, gdy utrata mitochondriów jest zbyt duża (ok. 40%), energii wystarcza już tylko do funkcjonowania organizmu w ograniczonym zakresie. Człowiek traci odporność, zaczyna łapać infekcje, jest chronicznie przemęczony.

Im większa liczba mitochondriów jest uszkodzona, tym organizm jest coraz słabszy, bo energii wystarcza już na funkcjonowanie tylko najważniejszych organów.

Pojawiają się różne schorzenia i dysfunkcje organizmu, które zazwyczaj medycyna leczy objawowo.

Terapia mitochondrialna polega na oddychaniu przy pomocy powietrza o naprzemiennie niskim i wysokim stężeniu tlenu.

Wiadomo, że ostra hipoksja, czyli niedotlenienie organizmu wiąże się ze szkodliwymi dla naszego organizmu skutkami.

Terapia mitochondrialna stymuluje własne endogeniczne mechanizmy obrony na wszystkich poziomach – od genu do całego organu lub tkanki. W wyniku hipoksji stare uszkodzone mitochondria obumierają, a odbudowują się nowe zdrowe, w znacznie szybszym tempie.

Zastosowanie terapii IHHT

**Schorzenia neurologiczne:** • Zespół przewlekłego zmęczenia, • Bezsenność, • Migrena i częste bóle głowy, • Zaburzenia w koncentracji, • ADHD, • Zespół Aspergera, • Autyzm, • Stwardnienie rozsiane, • Demencja, • Choroba Parkinsona, • Choroba Alzheimer, • Upośledzenia umysłowe.

**Schorzenia hormonalne:** • Tarczycza, • Cukrzyca, • Zaburzenia wzrostu.

**Problemy układu oddechowego:** • Niewydolność oddechowa, • POChP, • Bezdech senny.

**Choroby nerek i wątroby:** • Zaburzenia trawienia, • Biegunka/zaparcia, • Refluks, • Nadmierny apetyt, • Otyłość, • Schorzenia kardiologiczne, • Zaburzenia rytmu serca, • Kardiomiopatie, • Nadciśnienie, • Schorzenia reumatyczne, • Reumatoidalne zapalenie stawów, • Fibromialgia, • Schorzenia ogólnoustrojowe, • Borelioza, • Kwasica mleczanowa, • Twardzina układu, • Obniżone napięcie mięśniowe.

Jak przebiega terapia? Terapia mitochondrialna polega na wdychaniu niedotlenionego powietrza (małe stężenie tlenu) z przerwami na nadtlenione powietrze (wysokie stężenie tlenu). Fazy normoksyczne (normalne stężenie tlenu) mogą zastąpić fazy hiperoksyjne, ale są ogólnie mniej wydajne, ponieważ przywrócenie normalnych poziomów SpO<sub>2</sub> trwa dłużej, a efekt kolejnej fazy hipoksyjnej jest zmniejszony.

Pacjent wdycha przez maskę dokładnie kontrolowaną mieszankę powietrza dostarczaną przez urządzenie.

Podczas trwania całego zabiegu, pacjent pozostaje w wygodnej pozycji leżącej, często pacjent zasypia i opisuje całą procedurę, jako głęboko relaksującą.

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