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CORRECTION BY ANTIOXIDANTS OF OXIDATIVE ACTIVITY IN AN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE

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The advantage of the reserpine model in the development of parkinsonian syndrome in experimental animals was shown: symptoms appeared as early as 2 *h* after reserpine administration and persisted for up to 3 days. Injection of reserpine at a dose of 3 *mg/kg*, quite clearly influenced behaviour. Significant changes in biochemical indicators, markers of oxidative stress, accompanying disorders of motor functions of experimental animals were revealed. The possibility of correcting the pathology with the help of some agents of antioxidant nature has been shown. Despite the lack of clarity regarding the efficacy of succinate, tocopherol, the study showed that the use of goji berries significantly reduces the risk of Parkinson's disease.

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***Keywords*:** Parkinson's disease, oxidative stress, antioxidants, Lycium barbarum.

Introduction. Parkinson's disease (PD) is one of the most common neurodegenerative pathologies presenting a significant medical and socioeconomic challenge. This progressive neurodegenerative disease, with its subsequent development of severe disability, is of great interest to neurophysiologists, biochemists, and scientists from other medical fields due to its clinical heterogeneity and progressive course. There is active work on the study of possible mechanisms of neurodegeneration in PD: oxidative stress, mitochondrial disorders, environmental factors, aging, genetic factors are considered.

There are many publications on the role of the major gateway for electron entry into the respiratory chain (mitochondrial respiratory chain complex I) in the pathogenesis of PD. There is evidence that in PD there is improper assembly of this complex due to differential reduction of the complex I subunit, increased oxidative damage of the subunits and, as a result, decreased electron transfer rate through the complex [1]. There are studies indicating low complex I activity in less affected areas of the brain, suggesting an early pathogenetic process. Whether disturbances in the activity of the complex are the main defect leading to the development of PD, or whether they are secondary changes remains an open question [2].

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The role of oxidative stress in the pathogenesis of PD has been extensively discussed. Being more energetically demanding, neurons contain more mitochondria, hence produce more reactive oxygen species and may be particularly vulnerable to oxidative stress. It has also been shown that with age there is an increased metabolism of dopamine in the substantia nigra cells of the brain, which leads to increased production of reactive oxygen species, mitochondrial dysfunction, leading to reduced ATP formation, reduced degradation and recycling of "waste" proteins, which ultimately impairs normal cell function [3–5]. Moreover, it has been shown that the level of carbonyl derivatives of proteins (a marker of protein oxidation) is doubled in the substantia nigra of the brains of PD patients, and the level of lipid hydroperoxides (a marker of lipid oxidation) is ten times higher [6].

The causal links in the development of the disease have not been fully identified and medicine is seeking to identify lifestyle features that could potentially reduce the risk of developing PD, looking for molecular and biochemical markers of the disease suitable for early (before the onset of motor impairment) screening diagnosis of the disease [7]. Given that dopamine is oxidised to dopamine-quinones, which subsequently bind to and deactivate strong antioxidants (e.g. superoxide dismutase 2), it is logical to assume that reducing oxidative stress is a potentially effective way to prevent PD [8]. Indeed: preclinical studies of antioxidants in animal models of PD have yielded encouraging results. It was shown that rats that received antioxidant-rich food instead of regular food had fewer dopaminergic neurons dying after neurotoxin injection [9]. Several research groups have suggested that vitamin E, vitamin C or beta-carotene can reduce the risk of PD, but there are studies that indicate no association between these antioxidants and the risk of developing the pathology [10]. From other comprehensive meta-analyses, experts have concluded that vitamin E protects against PD [11], but found no link between potential disease prevention and carotenoids or vitamin C. Campolo et al. suggest that Parkinson's neurodegeneration is associated with a deficiency of antioxidant compounds in the diet and that deficiencies such as folic acid, vitamins (A, C, E, niacin) and selenium increase the risk of disease.

Thus, the efficacy of antioxidants as a therapy for PD remains under debate and the results are not fully elucidated and studied in humans. However, despite a weak evidence base, numerous studies have pointed to the potentially beneficial effects of antioxidant supplements and many research groups have suggested that a therapeutic approach to PD should include modulation of oxidative stress using antioxidants. Moreover, there is no drug in the arsenal of PD therapy whose neuroprotective effects have been unequivocally clinically proven.

Purpose of the work: to study the parameters of oxidative status in the brain tissue of rabbits with a model of experimental parkinsonism against the background of taking antioxidants of various mechanisms of action and to evaluate their biological effect.

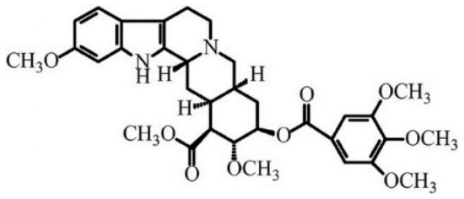
Materials and Methods.

Experimental Animals. The studies were carried out on domestic rabbits (*Oryctolagus cuniculus*), weighing 2.2–2.5 *kg*. Animals were kept in standard vivarium conditions with free access to water and food in a room with air

temperature of 22°C with a 12-hour light/dark cycle in accordance with the rules of bioethics approved by the National Bioethics Committee (Armenia).

Induction of Parkinson's Disease. In an experimental group of rabbits, a reserpine induced model of Parkinson's syndrome was achieved by a single injection of reserpine at a dose of 3 *mg*/*kg* subcutaneously (administration of this dose allows the more distinct manifestations of toxic effects to be observed). Reserpine was dissolved in glacial acetic acid (100 μL), then in water for injection at a ratio of 1:35 [12]. Previously, we found that when administering the above dose, the toxic effect of reserpine on the behaviour of rabbits was more clearly manifested than when administering smaller doses.

Reserpine is a central sympatholytic administered to animals, the systemic administration of which depletes dopamine in nerve endings. This approach makes it possible to detect neurological abnormalities characteristic of neurodegenerative diseases. The advantage of reserpine model is in fast development of parkinsonian syndrome: in 2–3 *h* after reserpine injection the neurological symptoms increase and last up to 3 days. This model can be used to study new anti-Parkinsonian substances when administered systemically [13]. The intact animals were injected with saline in the same volume.



Scheme. Structure of reserpine.

Experimental Design. After quarantine, rabbits were randomly divided into intact (n=4) animals that consumed a balanced common diet throughout the experiment and experimental (n=16) animals.

The duration of the experiment was 8 days. The first experimental group (I) were control animals with reproduced PD after quarantine. The other experimental groups were enriched with an appropriate antioxidant for 6 days after quarantine, after which parkinsonism was simulated and the parameters were examined after 2 days. The II group – animals received α-tocopherol at a dose of 25 *mg*/*kgbw*; the III group – received succinic acid (succinate) at a concentration of 0.01%; the IV group – received Chinese dried goji berry extract at a dose of 5 *g*/*kgbw* as a drink. The berries were steamed with distilled water in a 1:3 ratio at 50–55°C, infused for 3 *h* at 22±2°C.

Goji berries – fruits of shrub of genus *Lycium Barbarum* (*L. Barbarum*) are harvested in industrial quantities in North-West China in Ningxia, Gansu, Hebei, Qinghai provinces and in the Republic of Kazakhstan. Berries are used as an independent food additive and as components of functional products in dried (including freeze-dried) form, as well as in the form of condensed extracts.

Determination of Biochemical Parameters. Biochemical parameters have been determined in brain homogenates. The brain tissue has been homogenized in a Potter–Elvehjem glass homogenizer. To identify the antioxidant status of the organism, the activities of superoxide dismutase (SOD) and catalase have been determined. SOD activity has been determined by the rate of adrenaline autooxidation [14]. Catalase activity has been determined by the colorimetric method [15]. The intensity of lipid peroxidation has been determined by the level of its molecular product, malondialdehyde (MDA). MDA has been determined by the reaction with thiobarbituric acid spectrophotometrically at a wavelength of 535 *nm* [16]. The intensity of oxidative degradation of proteins has been assessed by the level of their carbonyl derivatives recorded in the reaction with 2,4-dinitrophenylhydrazine. The optical density of the resulting dinitrophenylhydrazones has been recorded at a wavelength of 270 *nm* [17].

Statistical Analysis. The data are presented as arithmetic values with standard error according to Microsoft Excel 2013. Standard error does not exceed 3% of absolute values. Statistical hypotheses were tested using Student's *t*-test [18], the difference between the results of different series of experiments was considered significant at $p < 0.05$.

Results and Discussion. Throughout the period of the experiment, the status of intact animals did not deviate from the physiological norm, and the body weight dynamics were positive.

Behavioural reactions in the experimental groups were monitored in order to determine the degree of disease progression. The activity of motor functions was recorded before the start of the experiment and then at various intervals after the administration of reserpine for 2 days.

In all animals of the experimental groups, except IV group animals, symptoms of impaired motor functions became apparent 3 *h* after the administration of reserpine. Worsening of the general state was noted: hunched posture, lethargy, uncoordinated actions of the limbs, which reliably indicated the development of pathology. Motor activity was observed in the "open field" test: the number of horizontal and vertical movements (stance) was recorded for 5 *min*. A rather pronounced decrease in motor activity (oligokinesia) was detected. On the 2nd day the severity of rigidity was evaluated, using the "hump" symptom, which was based on shortening of the distance from the neck to the base of the tail, using a three-point system. Rigidity was assessed with a score of 2.

Tremors of the limbs were detected in the same time interval. The tremor was assessed according to the severity (amplitude) and frequency of oscillations, recording the time of onset and termination in points (in a three-point system). Local mid-amplitude tremor was noted (2 points). Signs of catalepsy were observed, which was measured by the time the animal stiffened (in seconds) in an unfamiliar posture. The animal was seated on its hind legs so that while resting the front leg on the step, the animal would hold the other leg unsupported. Animals were frozen for 8–10 *s*, corresponding to a score of 3. Assessment of autonomic disturbances showed quite pronounced ptosis of the upper eyelids. Some results are shown in Table.

Assessment of impaired motor activity under the influence of reserpine

Severity of motor impairment						
Catalepsy, points			Ptosis, points			
weak, 1–2	moderate, 3–4	expressed, 5–6	eyes fully open, 0	a gap of up to 2 *mm*, 1	a gap of up to 2 *mm*, 2	total eye closure, 3
–	+	–	–	+	–	–

It should be noted that the greatest decrease in motor activity was observed on the 2nd day. On the 3rd day the pronounced changes were observed to subside and motor functions recovered, by the end of the day the indices did not differ from the background ones. Motor activity disorders were taken as evidence of disease development, and at the peak of pathological manifestations some biochemical indicators were determined.

The activity of antioxidant protection enzymes in brain tissue homogenates was analysed in the study: superoxide dismutase (SOD) and catalase activities were determined. Fig. 1 shows that the value of the activities of the enzymes of the control group is significantly lower compared with the intact group. In the control group on the 2nd day of the experiment, SOD activity in rabbit brain homogenates decreased by 33% and catalase activity by 24%, indicating the depletion of the endogenous antioxidant system, that is, the development of oxidative stress.

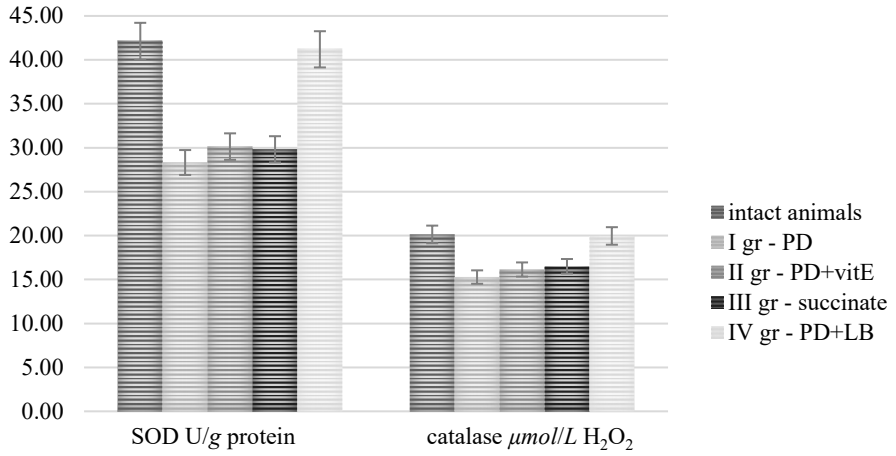


Fig. 1. Effect of antioxidants on the activity of antioxidant defense enzymes in rabbit brain homogenates under conditions of parkinsonian syndrome.

It is known that a consequence of an imbalance between oxidative and antioxidative processes is also increased lipid peroxidation. Evaluation of the values of the secondary product of lipid peroxidation, MDA, indeed showed an accumulation of MDA in brain tissues. In brain homogenates of intact animals, the concentration of MDA was 7.15 *μm*/*g*, in the control group it was 13.22 *μm*/*g* (Fig. 2). An 85% increase in dialdehyde concentration against the background of the development of the parkinsonian syndrome indicates a significant disturbance in the regulation of free-radical processes.

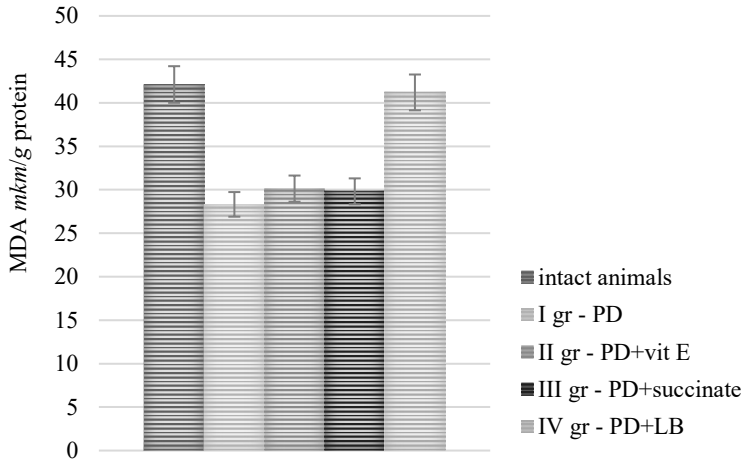


Fig. 2. Effect of antioxidants on the value of MDA, a marker of oxidative stress in rabbit brain homogenates under conditions of Parkinsonian syndrome.

Available data in the literature indicate that under the influence of reactive oxygen species changes in the structure of protein molecules occur, which disturb its physico-chemical and biological properties and protein oxidation characterizes the severity of "oxidative stress" in various pathological states. To reveal the degree of "oxidative deformation" of proteins, the concentration of protein carbonyl groups, markers of oxidative modification, was determined by interaction of protein carbonyl derivatives with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazones. The concentration of 2,4-dinitrophenylhydrazones in brain homogenates from PD animals increased by 57% compared to the intact group (Fig. 3). Increased levels of 2,4-DNP-hydrazones at 270 *nm* indicate increased intensity of spontaneous protein degradation.

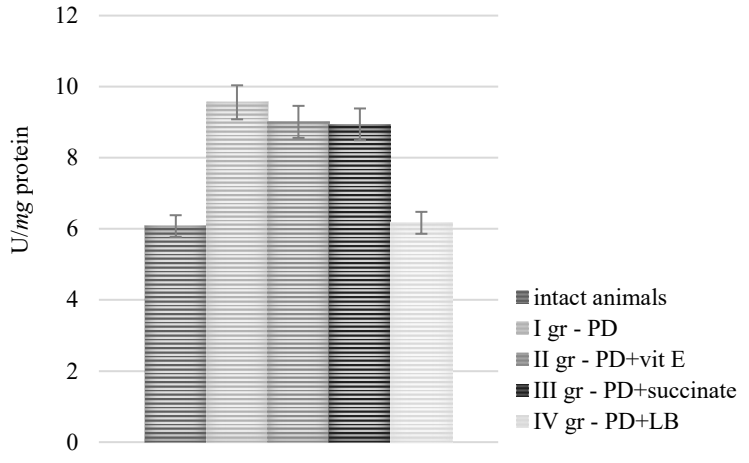


Fig. 3. Intensity of protein oxidation in brain homogenates under oxidative stress and as a result of antioxidants.

The findings suggest that systemic oxidative stress plays an important role in the molecular mechanisms of PD, and that reducing oxidative stress may be a potentially effective way to prevent PD. In light of the above, it is of interest to study the effects of antioxidants on the development of Parkinsonism. There are numerous studies on the search and development of antioxidant pharmacotherapy, which are conducted in two directions: the use of synthetic drugs and natural antioxidants. Our choice was based on the following factors: since the action of toxic agents can lead to failures in the electron transport chain and a subsequent increase in oxidative stress, we used succinate, and in view of the numerous studies on the potential beneficial effects of antioxidant supplements – the antioxidant vitamin E (α-tocopherol) and goji berry extract.

α-tocopherol is characterized by high antiradical activity due to its ability to interact directly with free radicals and to stabilize the lipid bilayer of membranes by forming strong complexes with polyunsaturated fatty acids of membrane lipids.

Goji berries and their extracts contain a sufficient number of phytonutrients with a wide range of pharmacological properties, including those affecting lipid metabolism, oxidative processes, inflammatory reactions associated with the accumulation of reactive oxygen species [19].

To study the severity of the pharmacological effects of the above drugs, the ration of experimental animals was prophylactically enriched with appropriate supplements for 6 days before the administration of the toxic agent and then for two days after the administration.

As a result, the following values of SOD and catalase activities were obtained in brain tissue homogenates of rabbits of the groups with the enriched diet. In the II group (with tocopherol), the activity of SOD was 28% lower than the activity of the intact group, but higher than that of the control I group (5%). Activity of catalase was lower than activity of the intact group by 20%, but higher than the control group values (by 6%). In the III group (with succinate), the same pattern of activity fluctuations was observed (Fig. 1).

The same dynamics were found in the next series of experiments. In the II and III groups the concentration of MDA was higher than in the intact group, but lower than in the I group (Fig. 2). Adding tocopherol to the diet (II group) did not stop the increase in the concentration of malonic dialdehyde (the value of the index was 79% higher than the intact value), but it slightly decreased relative to the control values (by 3%). In the III group, the concentration of MDA was lower than the control values by 2%, but higher than the intact value by 82%.

As a result of vitamin E and succinate administration, carboxyl group concentrations were higher, than in intact animals (by 47% and 48%, respectively), but lower (by 6% and 6%, respectively), than in control animals (Fig. 3).

The findings indicated that the effectiveness of these treatments was not convincing.

The introduction of goji berry extract into the diet changed the picture. The effectiveness of goji berry extract administration is shown in the same graphs. When berry extract was introduced into the diet of rabbits (IV group) on the 8th day of the experiment, the activity of both enzymes of antiradical protection almost repeated the values of intact animals. Compared to the data of control animals, the activity of

SOD increased by 97.8% and the activity of catalase by 99%. The IV group showed no accumulation of toxic MDA (MDA concentration was 65% lower than the control values). In the same group no increase of protein carboxyl groups was observed: approximately the same concentration as in intact homogenates was determined.

It should be noted that these biochemical data were expected, as the animals in this group showed practically no undesirable motor complications after reserpine administration.

Conclusion. Since dietary antioxidants, by capturing reactive oxygen species, can protect against neuronal damage, it is reasonable to consider the prophylactic use of natural preparations with antioxidant activity. The results indicate a relationship between the antioxidant capacity of the diet and the likelihood of disease development. The study revealed a pronounced pharmacological effect of the berry extract *L. Barbarum*. It was shown that the application of the extract reliably inhibited the oxidative activity, which is probably due to the high content of selenium, vitamins C, E, bioavailable minerals. The data indicate the advisability of using berries as part of the diet for the prevention and, possibly, the correction of brain pathologies.

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ՀԱԿԱՕՔՍԻԴԱՆՏՆԵՐՈՎ ՕՔՍԻԴԱՆՏԱՅԻՆ ԱԿՏԻՎՈՒԹՅԱՆ ՇՏԿՈՒՄԸ ՊԱՐԿԻՆՍՈՆԻ ՀԻՎԱՆԴՈՒԹՅԱՆ ՓՈՐՁԱՐԱՐԱԿԱՆ ՄՈԴԵԼՈՒՄ

Ցույց է տրվել փորձարարական կենդանիների օրգանիզմում պարկինսոնային ախտանիշների զարգացումը ռեզերպինային մոդելով. ախտանիշները ի հայտ են եկել ռեզերպինի ներարկումից 2 *ժ* անց և պահպանվել 3 օր: 3 *մգ/կգ* ռեզերպինի ընդունումը բավականին հստակ ազդեցություն է ունեցել կենդանիների վարքի վրա: Բացահայտվել են փորձարարական կենդանիների շարժողական խանգարումները ուղեկցող կենսաքիմիական ցուցանիշների՝ օքսիդային սթրեսի մարկերների զգալի փոփոխություններ: Ցույց է տրվել որոշ հակաօքսիդանտների օգնությամբ պաթոլոգիայի շտկման հնարավորությունը: Tocopherol-ի և succinate-ի արդյունավետությունը հստակ չի արտահայտվել, սակայն գոջի հատապտուղների օգտագործումը զգալիորեն նվազեցրել է Պարկինսոնի զարգացման ռիսկը:

Н. К. АЙРАПЕТЯН, И. А. БАДАЛЯН

КОРРЕКЦИЯ АНТИОКСИДАНТАМИ ОКИСЛИТЕЛЬНОЙ АКТИВНОСТИ В ЭКСПЕРИМЕНТАЛЬНОЙ МОДЕЛИ БОЛЕЗНИ ПАРКИНСОНА

Показано преимущество резерпиновой модели в развитии паркинсонического синдрома экспериментальных животных: симптомы возникали уже через 2 *ч* после введения резерпина и сохранялись до трех суток. Введение резерпина в дозе 3 *мг*/*кг* достаточно отчетливо повлияло на поведение. Выявились значительные изменения биохимических показателей – маркеров окислительного стресса, сопровождающие нарушения двигательных функций экспериментальных животных. Показана возможность коррекции патологии при помощи некоторых агентов антиоксидантного характера. Несмотря на отсутствие ясности в отношении эффективности сукцината, токоферола, исследование показало, что применение ягод годжи достоверно снижает риск развития болезни Паркинсона.