

THE COMBINED EFFECT OF *TRIFOLIUM PRATENSE* L. (RED CLOVER)
AND VIBRATION ON RAT BRAIN PROTEIN AMIDATION
DURING AGING

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Vibration pathologies are one of the pressing issues in medicine and biochemistry. It is known that under the influence of vibration, the content of amide groups of proteins in tissues increases, which is one of the biochemical indicators of the organism. It has been shown that amidation occurs to a lesser extent in brain proteins of old rats exposed to vibration than in young rats. When *Trifolium pratense* L. flowers are added to the animals' diet, the level of amidation during vibration in old rats also decreases to a lesser extent than in young rats. However, in both cases, the level of amidation occurring is reduced by half under the influence of the plant. Based on these results, the use of *T. pratense* plant material is proposed as a means of reducing the negative impact of vibration on the body.

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Keywords: *Trifolium pratense* L., vibration, amidation, amide groups of proteins.

Introduction. Numerous studies have shown that exposure to extreme environmental factors leads to changes in the body's biochemical parameters, among which the amide groups of tissue proteins play a significant role. It has been established that factors that induce excitatory processes lead to deamidation of proteins in tissues, while, conversely, protein amidation occurs during the development of inhibitory processes [1, 2].

Among the adverse environmental factors affecting the body, vibration occupies a special place, being a stressor for humans and animals. In addition to changes in protein amidation [2, 3], vibration also causes oxidative stress, initiating lipid peroxidation [4, 5]. It is also worth noting that vibration pathology ranks second among diseases associated with professional activity.

Even mild vibration exposure is known to be a powerful stressor for the central nervous system, causing intense bioelectrical changes in the brain and peripheral damage to the vestibular system. Long-term vibration exposure can cause vibration syndrome, which affects the nervous, cardiovascular, motor, and other body systems. However, short-term vibration therapy is used to treat eye diseases, asthma, and other conditions [6].

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There are various ways to adapt to stress, including the use of herbal preparations. The antihypoxic effect of the plant *T. pratense* on the dynamics of protein amide groups during hypobaric hypoxia has previously been demonstrated [7]. This plant also exhibits antioxidant activity against lipid peroxidation in rat tissues exposed to hypoxia [8, 9]. A study of the effects of vibration on brain protein amidation also revealed an effect of the plant *T. pratense* on vibration-induced amide group dynamics [3].

Thus, it was shown that the plant *T. pratense* has complex antihypoxic, antioxidant, and stress-protective properties, which are apparently explained by the various biologically active substances contained in the plant, in particular, isoflavonoids and phenols [8, 10, 11]. A high content of phenols and flavonoids is noted in the flowers of the plant, as well as a correlation between the content of these substances and the antioxidant activity of plants [12].

In this present work, the amidation level of brain proteins in old rats under vibration conditions has been studied. Taking into account the stress-protective properties of *T. pratense* plant, the influence of the plant on amidation processes has also been studied during the aging of rats.

Materials and Methods.

Animals and Plant Materials. All experiments were carried out on Wistar rats (young group – 4–6 months, and old group – 24 months) in accordance with the current ethical norms stated by “International Recommendation on Carrying out of Biomedical Researches with Use of Animals” and the study plan has been approved by the National Center of Bioethics (Armenia). The animals were kept under standard conditions of a vivarium (temperature $22 \pm 2^\circ\text{C}$ in a light/dark cycle of 12 h).

The first group of rats had a standard vivarium diet, the second group had a diet with – the addition of *T. pratense* dry flowers of 0.5 g of crushed plant material per 100 g of animal weight for 10 days. Plants were collected near Hankavan (Kotayk Province, Armenia).

Vibration was realized on the ST-300 vibrostent at the Chair of Human and Animal Morphology and Physiology of the YSU. Vibration frequency was 60 Hz, deviation amplitude was equal to 1.0 mm. The whole organism of the animal was exposed to vibration for 2 h daily for 5 days.

Biochemical parameters have been determined in the brain homogenates. The brain tissue has been homogenized in a Potter-Elvehjem glass homogenizer at $+4^\circ\text{C}$ by 0.05 M potassium phosphate buffer (pH 7.4).

Amide Group Assays. Amide groups of brain proteins were determined by the method of harsh acid hydrolysis in pre-cleaned sediments of brain homogenates [13] by the amount of released ammonia in the hydrolysate using Silakova’s method [14]. Proteins were hydrolyzed in 1 N H_2SO_4 for 180 min in order to determine the total content of amide groups.

Data Processing. Statistical analysis of the data was performed using Student’s *t*-test, Fisher’s test, and the liability method [15]. A difference of $p < 0.05$ or less in the mean values was considered as statistically significant.

Results and Discussion. Brain protein amidation was studied in aged rats under normal conditions and under vibration. According to the data obtained, the amide group content in brain proteins of young rats (4–6 months) is normally

29.88 $\mu\text{mol/g}$ (Fig. 1). Under the influence of 2-hours vibrations over 5 days, the amide group content increased almost twofold to 58.50 $\mu\text{mol/g}$, which is completely consistent with our previous studies [3].

In the brain of old rats (24 months), the amount of protein amide groups normally decreases to 24.75 $\mu\text{mol/g}$, i.e. their quantity decreases by 5.13 $\mu\text{mol/g}$ compared to young individuals. Protein amidation also occurs under the influence of vibration, but to a significantly lesser extent than in young rats: the amount of amide groups in the brain of old rats increases to 31.7 $\mu\text{mol/g}$, i.e., by 6.95 $\mu\text{mol/g}$ compared to the norm (Fig. 1).

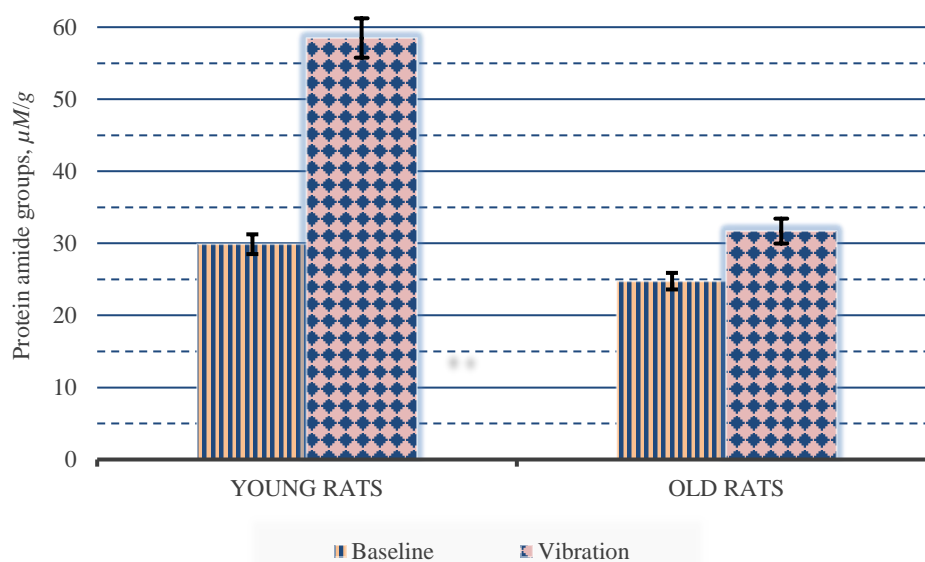


Fig. 1. Amide nitrogen content of young and old rat brain proteins subjected to vibration ($\mu\text{mol/g}$ fresh tissue; $n = 4$, $p < 0.05$).

Thus, brain proteins from old rats contain fewer amide groups, and vibration-induced amidation is also reduced compared to protein amidation in young individuals.

In this study, the effect of *T. pratense* on the amidation of brain proteins in old rats exposed to vibration was also investigated. The flowers of the plant were added to the animals' feed for 10 days at a rate of 0.5 g per 100 g of body weight per day, after which the animals were exposed to 2-hour vibration for 5 days, during which time the flowers of the plant were continued to be added to the feed. The results are shown in Fig. 2.

The increase of brain proteins amidation induced by vibration descends twofold in addition to the plant *T. pratense* in young rats, as shown in Fig. 2. When the plant was added to the animals' diet, the level of amidation of brain proteins in young rats exposed to vibration (58.50 $\mu\text{mol/g}$) significantly decreased and amounted to 45.12 $\mu\text{mol/g}$, which was also previously established by our studies [3]. The same dynamics of brain protein amidation descend has been observed in old

rats. The level of brain proteins amidation in old rats ($31.7 \mu\text{mol/g}$) decreased to a lesser extent, to $28.49 \mu\text{mol/g}$.

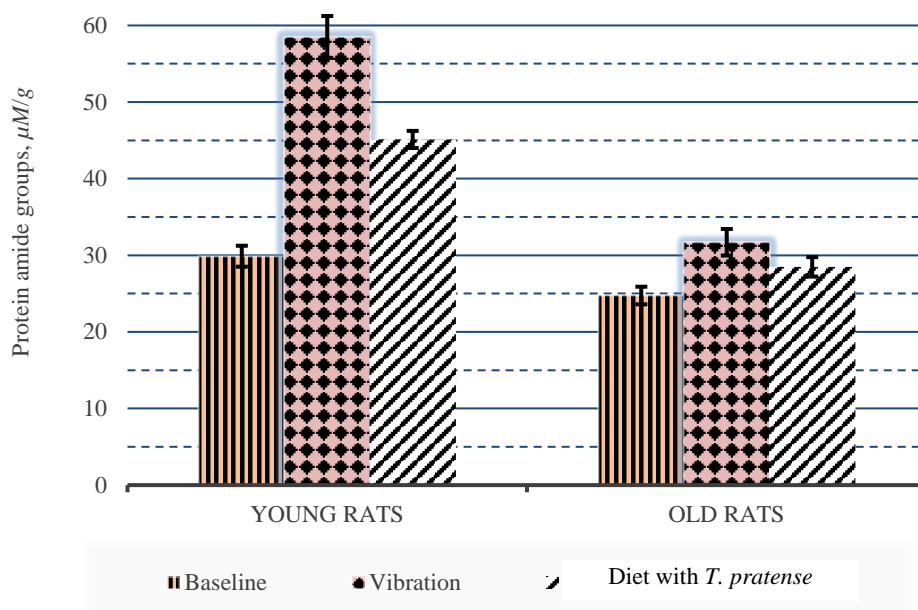


Fig. 2. Effect of *T. pratense* on the dynamics of amide group changes in brain proteins of vibration-exposed rats ($\mu\text{mol/g}$ fresh tissue; $n = 4$; $p < 0.05$).

However, it should be noted that the stress-protective properties of *T. pratense* plant reduce vibration-induced amidation of brain proteins to the same extent in both young and old rats.

Conclusion. Based on the analyses of the obtained results and literature data, we can conclude that the content of amide groups in rat brain proteins decreases during aging. In old rats brain protein amidation takes place under the influence of vibration, but to a significantly lesser extent compared to young rats.

Under the influence of the plant *T. pratense*, in young and old rats exposed to vibration, the level of amidation of brain proteins decreases by approximately 50% compared to the amidation that occurs without the addition of the plant.

Stress-protective properties of *T. pratense* plant may be used to reduce the negative impact of vibration on the organism.

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TRIFOLIUM PRATENSE L. (ԵՐԵՔՆՈՒԿ ՄԱՐԳԱԳԵՏՆԱՅԻՆ) ԲՈՒՅՄԻ
ԵՎ ՎԻԲՐԱՑԻՎԱՅԻ ՀԱՄԱԼԻՐ ԱԶԳԵՑՈՒԹՅՈՒՆԸ ԱՌՆԵՏՆԵՐԻ
ԳԼՍՈՒՂԵՂԻ ՍՊԻՏԱԿՈՒՑՆԵՐԻ ԱՄԻԴԱՑՄԱՆ ՎՐԱ
ԾԵՐԱՑՄԱՆ ԺԱՄԱՆԱԿ

Վիբրացիոն պաթոլոգիաները դասվում են բժշկության և կենսաքիմիայի արդիական խնդիրների շարքին: Հայտնի է, որ վիբրացիայի ազդեցությամբ

մեծանում է սպիտակուցների ամիդային խմբերի պարունակությունը հյուսվածքներում, որն օրգանիզմների կենսաքիմիական ցուցիչներից մեկն է հանդիսանում: Ցույց է տրված, որ ծեր առնետների ուղեղի սպիտակուցներում վիրրացիայով պայմանավորված ամիդացման աստիճանն ավելի ցածր է, ի համեմատ երիտասարդ առնետների: Կենդանիների սննդակարգին *Trifolium pratense* L. բույսի ծաղիկներ ավելացնելիս, ծեր առնետների ուղեղում ամիդացման մակարդակը նույնպես նվազում է ավելի քիչ չափով, քան երիտասարդ առնետների մոտ: Սակայն, պետք է նշել, որ երկու դեպքում էլ բույսի ազդեցությամբ տեղի ունեցող ամիդացման աստիճանը կիսով չափ կրճատվում է: Այս արդյունքների հիման վրա առաջարկվում է *Trifolium pratense* L. բույսի օգտագործումը՝ որպես վիրրացիայի բացասական ազդեցության նվազեցման միջոց:

И. А. БАДАЛЯН, А. А. ПОЛАДЯН

КОМПЛЕКСНОЕ ВЛИЯНИЕ РАСТЕНИЯ *TRIFOLIUM PRATENSE* L.
(КЛЕВЕР ЛУГОВОЙ) И ВИБРАЦИИ НА АМИДИРОВАННОСТЬ
БЕЛКОВ ГОЛОВНОГО МОЗГА ПРИ СТАРЕНИИ КРЫС

Вибрационные патологии являются одной из актуальных проблем медицины и биохимии. Известно, что под воздействием вибрации происходит повышение содержания в тканях амидных групп белков, которые являются одним из биохимическим показателей организма. Показано, что в белках головного мозга старых крыс при вибрации происходит амидирование в меньшей степени, нежели у молодых крыс. При добавлении в рацион животных цветков растения *Trifolium pratense* L. уровень амидированности при вибрации у старых крыс спускается также в меньшей степени, чем у молодых крыс. Однако в обоих случаях под влиянием растения уровень происходящего амидирования уменьшается в 2 раза. На основании полученных результатов предлагается использование растительного материала *Trifolium pratense* L. как средства снижения отрицательного воздействия вибрации на организм.