PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2025, **59**(1–2), p. 36–42

Biology

EFFECT OF THE PLANT *TRIFOLIUM PRATENSE* L. ON AMIDATION OF BRAIN PROTEINS IN RATS EXPOSED TO VIBRATION

I. A. BADALYAN [*], N. K. HAYRAPETYAN [**], A. A. POLADYAN [***]

Chair of Biochemistry, Microbiology and Biotechnology, YSU, Armenia

It has been shown that under vibration conditions there is significant amidation of rat brain proteins, which is characteristic of inhibition processes. When studying the effect of *Trifolium pratense* L. (Red Clover) plants on the amidation of proteins under vibration conditions, it was shown that adding dry flowers of the plant to the animals' diet leads to noticeable decrease in the amidation of brain proteins. The study showed that the *T. pratense* plant has a stress-protector effect under vibration conditions. Based on the conducted research, it is proposed to use the plant *T. pratense* in the development of broad-spectrum anti-stress drugs.

https://doi.org/10.46991/PYSUB.2025.59.1–2.036

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

Introduction. One of the main properties of a living system is the ability to respond to external influences by the excitation process. One of the fragments of the chemistry of this process is the formation and elimination of ammonia in the tissues of the organism. Contrary to the opinion that ammonia is a “metabolic waste”, ammonium ions participate in complex biochemical processes of the organims. Ammonia can be produced by the following compounds: the glutamine – glutamic acid system, the adenylic system (ATP–AMP), as well as amide groups of proteins. It has been shown that changes in the amidation of tissue proteins depend on the effects of extreme environmental factors and the functional state of the organism caused by them. It has been also established that with the predominance of excitation processes in the brain of animals, deamidation of proteins occurs. Thus, according to some data in literature, with electrical stimulation, hypoxia, with the introduction of insulin and camphor into the organism, amide groups of brain proteins are broken down. During the development of inhibitory processes during natural sleep, hibernation, cold acclimatization, and drug-induced sleep, on the contrary, amidation of brain proteins occurs [1]. An increase in amidation of brain proteins has also been shown in the organism exposed to vibration [2].

Vibration, which is a significant stress factor for humans and animals, is one of the pressing problems of medical biology. It is known that an increase in the frequency of vibrations and their duration leads to the development of vibration

[*] E-mail: irinabadalyan@ysu.am
[**] E-mail: nn.hayrapetyan@ysu.am
[***] E-mail: apoladyan@ysu.am

disease [3, 4], but short-term vibration therapy is used in the treatment of asthma, cardiovascular, eye and other diseases [5].

Vibration causes the formation and accumulation of active forms of oxygen, which causes tissue hypoxia in the organism [6]. Hypoxia, in turn, can cause metabolic disorders that lead to structural damage to cells [7].

Under hypoxic conditions, biological oxidation processes are suppressed, resulting in a decrease in the amount of ATP and creatine phosphate, which causes an energy deficiency [8]. It is known that amide groups of tissue proteins can serve as an alternative to energy resources. According to Dobry and Sturtevant, the amide bond contains approximately 5840 *kcal*, which is a semi-macroergic bond and can be used in conditions of ATP deficiency. Thus, according to Veksler, the energy released during the rupture of amide groups can ensure the synthesis of ATP and creatine phosphate when the organism's main energy resources are depleted [9]. It should be noted that the concentration of amide bonds in nervous tissue is significantly higher than the concentration of macroergic phosphorus, and thus the potential reserves of this energy exceed the energy of macroergs [9]. Thus, by studying the dynamics of amide groups of tissue proteins under various stressful influences, one can also judge the expenditure or accumulation of energy in amide bonds.

It is known that plants containing various biologically active substances are used for the prevention and treatment of stress-related disorders. In particular, *T. pratense* contains isoflavonoids, genistein, daidzein and other phenols with antioxidant activity. It has been shown antihypoxic and antioxidant effects of *T. pratense* on the organism under hypoxic conditions [10–12].

The purpose of the work was to study the effect of vibration on the amidation of rat brain proteins, as well as the effect of the plant *Trifolium pratense L.* (Red Clover) on the dynamics of amide groups during vibration.

Materials and Methods.

Animals and Plant Materials. All experiments were carried out on Wistar rats (weight 100–150 *g*) 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 vivarium (temperature 22 ± 2°C in a light/dark cycle of 12 *h*).

The first group of rats had usual 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 Physiology of the YSU. Vibration frequency was 60 *Hz*, deviation amplitude was equal to 1.0 *mm*. The animal whole organism was exposed to vibration 2 *h* daily during 5, 10 and 15 days.

Biochemical parameters have been determined in the brain homogenates. The brain tissue has been homogenized in a Potter-Elvehjem glass homogenizer at +4°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 the 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, and for 30 *min* for easily hydrolyzable amide groups. Hardly hydrolysable amide groups were determined by the difference between the total and easily hydrolyzable amide groups.

***Data Processing*.** Statistic treatment of data was realized using Student Fisher differences and liability method [15]. A difference of $p<0.05$ or less in the mean values was considered as statistically significant.

Results and Discussion. The amidation of proteins in the rat brain was studied under normal conditions and under the influence of vibration. According to the obtained results, in baseline the total content of amide groups of proteins is 29.88 *μmol/g*, where 17.37 *μmol/g* are easily hydrolyzable amide groups of proteins, and 12.51 *μmol/g* – hardly hydrolyzable amide groups, respectively. Obtained results are shown in Fig. 1.

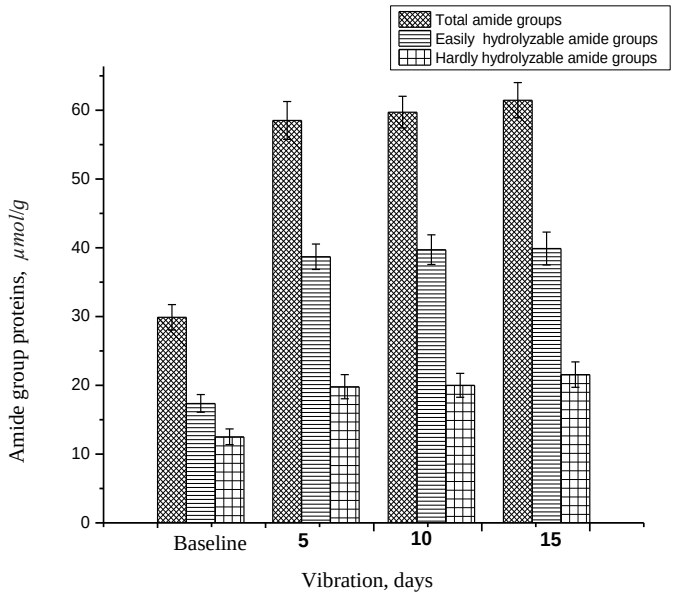

Fig. 1. Amide nitrogen content of rat brain proteins subjected to vibration (*μmol/g* fresh tissue; $n = 4$; $p < 0.05$).

Under the influence of 5-day vibration, a significant increase in amide groups of brain proteins is observed. Thus, the total content of amide groups increased almost twofold and amounted to 58.50 *μmol/g*. The amount of easily hydrolysable amide groups also increased twofold (38.70 *μmol/g*). There was some increase in the hardly hydrolysable amide groups (19.80 *μmol/g*). As can be seen from the presented data, the amidation of proteins under the influence of vibration occurs due to the easily hydrolyzable fraction of proteins.

It is obvious that with prolonged exposure to the same stimulus (5-day vibration), transition of stimulation to extreme inhibition takes place, which explains the significant amidation of proteins characteristic of inhibitory processes. According to literature data, the ongoing amidation of proteins is coupled with the accumulation of semi-macroergic bonds [9].

Longer exposure to vibration (10 and 15 days) does not result in further significant changes in protein amidation. In rats exposed to vibration for 10 days, the total amide groups are 59.72 *μmol*/*g*, i.e. there is insignificant amidation of proteins by 1.22 *μmol*/*g*. With a vibration duration of 15 days, amidation also increases slightly (by 1.74 *μmol*/*g*), compared to the effect of 10-day vibration. It is obvious that the organism develops an adaptation to the stress caused by five-day vibration, and further longer exposure (10–15 days) no longer causes a noticeable change in the content of amide groups of brain proteins. Further studies were conducted on rats subjected to 5-day vibration.

In order to reduce the negative impact of stress factors on the organism, the herbal preparations are widely used. Our early studies showed the antihypoxic and antioxidant effects of the *T. pratense* plant [10–12].

In this work, the effect of *T. pratense* on the amidation of rat brain proteins under vibration conditions was investigated. For this purpose, *T. pratense* flowers were added to the animals' feed at a rate of 0.5 *g* per 100 *p* of animal weight per day during 10 days, after which the rats were subjected to two-hour vibration for 5 days, while continuing to add the plant to the feed. The obtained results are presented in Fig. 2.

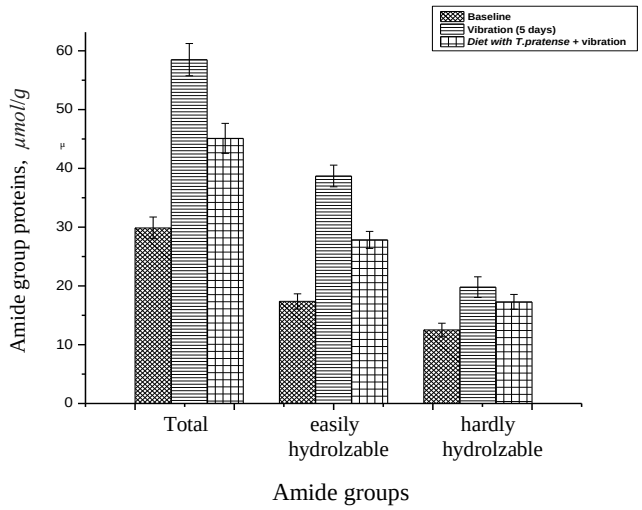

Fig. 2. Effect of *T. pratense* plant on the dynamics of amide groups of brain proteins in rats exposed to vibration (*μmol*/*g* fresh tissue; $n = 4$; $p < 0.05$).

According to the obtained results, under the influence of the plant *T. pratense*, there is a decrease in the amidation of brain proteins caused by vibration.

Thus, the content of total amide groups of proteins when the plant is added to the diet of rats decreases from 58.50 *μmol/g* to 45.12 *μmol/g,* that is, by 13.38 *μmol/g*. A decrease in amidation is also observed in the easily hydrolyzable fraction of proteins from 38.70 *μmol/g* to 27.83 *μmol/g* (by 10.87 *μmol/g*), and an insignificant decrease in the hardly hydrolyzable fraction – from 19.80 *μmol/g* to 17.29 *μmol/g* (by 2,51 *μmol/g*). It is obvious that the amidation of proteins that occurs during vibration, as well as the decrease in the level of amide groups under the influence of the plant, occurs due to the easily hydrolyzable fraction of proteins.

Based on the obtained results, it can be assumed that the *T. pratense* plant has not only antihypoxic and antioxidant properties [10–12], but also has a protective effect on the organism under the stressful effects of vibration.

Thus, the plant *T. pratense* can be recommended as an effective "universal stress-protector" in conditions of complex impact of unfavorable factors on the organism.

Conclusion. Based on the analysis of obtained results and the literature data, we can conclude that significant increase of amidation of brain proteins in rats exposed to vibration is reduced under the influence of the *T. pratense* plant. Significant changes in protein amidation both under vibration conditions and under the influence of the *T. pratense* plant occur mainly in the easily hydrolyzable fraction of amide groups.

It can be stated that the *T. pratense* plant has not only antihypoxant and antioxidant properties, but also has a stress-protective effect under vibrations.

This work can serve as a starting point for further studies of the influence of the plant *T. pratense* on various stress-related processes occurring in the organism of animals.

Based on the conducted study, it is proposed to use the plant *T. pratense* in the development of broad-spectrum anti-stress drugs.

Received 25.03.2025
Reviewed 19.05.2025
Accepted 25.05.2025

REFERENCES

1. Badalyan I.A., Dilbaryan A.S., Davtyan M.A. Amidation of Rat Brain Proteins During Hypoxia. *Reports of NAS RA* **113** (2013), 391–395 (in Russian).
2. Simonyan L.P., Papyan K.M., Badalyan I.A. Influence of Vibration on Ammonium Fermentation in the Rat Brain. *Mater. of the Sec. Republican Youth Sci. Conf.* (2001), 146–153 (in Russian).
3. Antoshina L.I., Saarkoppel L.M., Pavlovskaya N.A. Influence of Vibration on Biochemical Values Characterizing Oxidative Metabolism, Immunity, Metabolism in Muscular and Connective Tissues. *Labor. Med. and Industr. Ecol.* **2** (2009), 32–37 (in Russian).

4. Pacurari M., Waugh S., Krajnak K. Acute Vibration Induces Peripheral Nerve Sensitization in a Rat Tail Model: Possible Role of Oxidative Stress and Inflammation. *Neuroscience* **398** (2019), 263–272. https://doi.org/10.1016/j.neuroscience.2018.12.010
5. Likhachev S.A., Rushkevich Y.N. Influence of Proprioceptive Signals from the Neck Muscles on the Organization of Vestibuloocular Reactions. *Human physiology* **30** (2004), 685–688 (in Russian).
6. Vorobieva V.V., Shabanov P.D. Tissue-Specific Peculiarities of Vibration-Induced Hypoxia in Rabbit Liver and Kidney. *Bull. Exp. Biol. Med.* **167** (2019), 621–623. https://doi.org/10.1007/s10517-019-04583-0
7. Li H., Lantz R., Du D. Vibrational Approach to the Dynamics and Structure of Protein Amyloids. *Molecules* **24** (2019), 186. https://doi.org/10.3390/molecules24010186
8. Tkachenko E.A., Dekho M.A., et al. Adaptive Changes in the Activity of Enzymes in the Body of Mice under Oxidative Stress. *Bulletin of Veterinary Medicine* **65** (2013), 65–69 (in Russian).
9. Haroutunian A.V. The Pathways of Ammonium Formation and Neutralization in Brain. P*roblems of Brain Biochemistry. Academy of Sciences of Armenia* **2** (1966), 152–173 (in Russian).
10. Karapetyan M.A., Adamyan N.Yu. Badalyan I.A., Davtyan M.A. Influence of *Trifolium pratense* on Neurophisiological Processes and Amidation of Rat Brain Proteins under Hypoxic Conditions. *Materials of the Conference "Physiological Mechanisms of Regulation of the Organism's Activity"*. Yerevan (2012), 180–185 (in Russian).
11. Badalyan I.A., Trchounian A.A. The effect of *Trifolium pretense* Plant (Red Clover) on Lipid Peroxidation Processes in Rat Brain and Liver Exposed to Hypobaric Hypoxia. *Biolog. J. Armenia* **2** (2018), 39–43 (in Russian).
12. Badalyan I.A., Poladyan A.A. The effect of antioxidant activity of plants *Trifolium pretense L.* and *Crataegus laevigata* on lipid peroxidation in rat's tissues exposed to hypobaric hypoxia. *Proc. of the YSU. Chem. and Biol. Sci.* **56** (2022), 239–244. https://doi.org/10.46991/PYSU:B/2022.56.3.239
13. Pushkina I.V., Lukash A.I. Easy and Hard Hydrolysable Amide Groups in Protens. *Izv. Severo-Kavkaz. Nauch. Tsentra Vyssh. Shk. Yestestv. Nauki* **2** (1976), 95–97 (in Russian).
14. Silakova A.I., Trush G.P., Iaviliakova A. A Micromethod of Ammonia and Glutamine in Trichloroacetic Acid Tissue Extracts. *Voprosy Meditsinskoi Khimii* **8** (1962), 538–544 (in Russian).
15. Welham S.J., Gezan S.A., et al. Statistical Methods in Biology: Design and Analysis of Experiments and Regression. *CRC Press* (2015), 592.

Ի. Ա. ԲԱԴԱԼՅԱՆ, Ն. Կ. ՀԱՅՐԱՊԵՏՅԱՆ, Ա. Ա. ՓՈԼԱԴՅԱՆ

TRIFOLIUM PRATENSE Լ. ԲՈՒՅՍԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ՎԻԲՐԱՑԻԱՅԻ ԵՆԹԱՐԿՎԱԾ ԱՌՆԵՏՆԵՐԻ ԳԼԽՈՒՂԵՂԻ ՍՊԻՏԱԿՈՒՑՆԵՐԻ ԱՄԻԴԱՑՄԱՆ ՎՐԱ

Ցույց է տրված, որ վիբրացիայի ենթարկված առնետների մոտ տեղի է ունենում գլխուղեղի սպիտակուցների զգալի ամիդացում, ինչը բնորոշ է արգելակման գործընթացներին: Ամիդացման գործընթացների վրա *Trifolium pratense* L. (երեքնուկ մարգագետնային) բույսի ազդեցությունն ուսումնասիրելիս, պարզվեց, որ բույսի չորացված ծաղիկները կենդանիների կերին ավելացնելիս նկատվում է սպիտակուցների ամիդացման աստիճանի շոշափելի նվազում: Այս աշխատանքը ցույց է տալիս, որ *T. pratense* բույսը ցուցաբերում է հակասթրեսային ազդեցություն՝ կենդանիներին վիբրացիայի ենթարկելիս: Իրականացված հետազոտության հիմման վրա առաջարկվում է *T. pratense* բույսի կիրառումը հակասթրեսային պրեպարատների մշակման գործընթացում:

И. А. БАДАЛЯН, Н. К. АЙРАПЕТЯН, А. А. ПОЛАДЯН

ВЛИЯНИЯ РАСТЕНИЯ *TRIFOLIUM PRATENSE* L. НА АМИДИРОВАННОСТЬ БЕЛКОВ ГОЛОВНОГО МОЗГА КРЫС ПРИ ВИБРАЦИИ

Показано, что в условиях вибрации происходит значительное амидирование белков головного мозга крыс, свойственное процессам торможения. При исследовании влияния растения *Trifolium pratense* L. (клевер луговой) на амидированность белков в условиях вибрации выяснилось, что при добавлении в рацион животных сухих цветков растения происходит заметное уменьшение амидированности белков головного мозга. Исследование показало, что *T. pratense* оказывает эффект стресс-протектора в условиях вибрации. На основании проведенного исследования предлагается использовать клевер луговой в разработке антистрессовых препаратов широкого действия.