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THE EFFECT OF ANTIOXIDANT ACTIVITY OF PLANTS *TRIFOLIUM PRATENSE* L. AND *CRATAEGUS LAEVIGATA* ON LIPID PEROXIDATION IN RAT'S TISSUES EXPOSED TO HYPOBARIC HYPOXIA

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The processes of lipid peroxidation (LPO) were studied in rat brain and liver tissues under normal conditions, as well as under conditions of hypobaric hypoxia, since it is known that hypoxia causes the development of LPO in the cells of organism tissues. It has been also studied the influense of dry flowers of *Trifolium pratense* L. (red clover) and *Crataegus laevigata* (midland hawthorn) on lipid peroxidation, which triggers by hypobaric hypoxia. It has been shown, that the end product of lipid peroxidation malondialdehyde in rat brain and liver tissues was significantly decreased with the addition of plants flowers to the diet of animals, especially in the case of *C. laevigata*.

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***Keywords*:** hypobaric hypoxia, lipid peroxidation, *Trifolium pratense* L. (red clover), *Crataegus laevigata* (midland hawthorn).

Introduction. One of the most current problems of modern biology is the impact of stress on living organisms, as well as the study of possible ways to prevent pathological consequences caused by stress.

It has been shown the formation of free radicals in the living cells under stress conditions. Free radicals might be involved in various reactions causing some disorders in biochemical processes, which leads to cellular injury, and inhibition of enzymatic systems, which eventually may lead to the cell or even organism death [1, 2].

Among the adverse effects of the environment on living organisms, hypoxia is one of the most common and significant stress factors for humans and animals. Hypoxia causes several pathologies, among which should be mentioned intracellular oxidative stress, which promotes a significant increase in free-radical processes of lipid peroxidation (LPO). Initiation of the LPO has been shown during severe forms of hypoxia/ischemia with subsequent reoxygenation, as well as at hypobaric (altitude) hypoxia [3–5].

The level of lipid peroxidation normally contributes to maintaining the structural integrity of membranes, as well as the normal functioning of transport and enzymatic systems. However, during the pathological processes the activation of the LPO takes place leading to a misbalance between pro- and antioxidant systems. As

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a result, antioxidant cellular defense (AOD) is disturbed including enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (cGPx). Thus, LPO initiates the activation of post-stress and neurodegenerative pathologies [1, 4, 6, 7].

There are various ways to adapt to hypoxia, one of which is antioxidant and anti-hypoxante therapy. The antihypoxic effects inherent in chemical compounds are detected in many plants, including red clover (*Trifolium pratense* L.) flowers which contain isoflavones genistein, daidzein, and other phenols with high antioxidant activity [8–11].

In our previous studies, we have shown that under the influence of the *T. pratense* L. plant, there is a decrease in the LPO level in the tissues of rats under conditions of high-altitude hypoxia, which confirms the antioxidant properties of this plant [3].

This work aimed to study the effect of flowers of the plant *Crataegus laevigata* (midland hawthorn) on the dynamics of lipid peroxidation under conditions of hypobaric hypoxia (7.5–8.0 *km* above sea level), as well as a comparative analysis of the effect of these plants on the level of lipid peroxidation during hypoxia.

Materials and Methods.

Animals and Plant Materials. All experiments were carried out on Wistar (weight 100–150 *g*) rats 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℃ 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* L. dry flowers and the third one – diet with the addition of *C. laevigata* 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).

Hypoxic conditions were created in a hypobaric chamber with a pressure of 300 *mm* Hg, corresponding to a height of 7.5–8.0 *km*, with exposure of animals for 30 *min*. The animals were decapitated after exposure to hypoxia, the brain and liver were removed, and a 10% homogenate was prepared in 1.2% KCl.

Biochemical Analysis. The intensity of lipid peroxidation in the tissue homogenate is detected by the content of malonic di-aldehyde (MDA) in the reaction with 2-thiobarbituric acid by following formula [12, 13]:

$$C = \frac{D}{\varepsilon \cdot l},$$

where C is concentration of the MDA; ε is molar absorption coefficient in $1.56 \cdot 10^{-5}$ $M^{-1} \cdot cm^{-1}$; l is optical path length OPL, which corresponds to 1 *cm* of cuvette width.

Analytical tests were conducted using an UV-VIS spectrophotometer (Genesys 10S, USA) at λ=532 *nm* wavelength.

The MDA content is expressed in units of concentration ($\mu M/g$) of tissue.

Data Processing. All values were expressed as ± standard error of the mean. Data processing was done using Statistica 6.0 software. The differences between the

results of different series were considered valid, if Student criteria (p) was less than 0.01. A difference of $p<0.01$ or less in the mean values was considered as statistically significant.

Results and Discussion. The processes of lipid peroxidation in the brain and liver of rats in normal conditions and under conditions of high-altitude (7.5–8.0 *km* above sea level) hypoxia were studied by the content of the end product POL–MDA. According to the obtained results (Tab. 1), the normal content of MDA in the brain is 12.65 $\mu M/g$, which is almost 2 times higher than its level in the liver (6.40 $\mu M/g$). Under conditions of hypoxia, the accumulation of MDA sharply increases: in the brain – up to 53.85 $\mu M/g$, in the liver – up to 36.85 $\mu M/g$. Obtained results are consistent with the literature [1, 4, 14, 15].

Table 1

Effect of hypoxia on LPO processes in rat tissues (n=4, p<0.01)

Experiment condition	MDA content, $\mu M/g$ tissue	
	brain	liver
Baseline	12.65 ± 1.50	6.40 ± 0.75
Hypoxia	53.85 ± 4.20	36.85 ± 3.62
Difference	41.2	30.5

It should be mentioned that the content of MDA in the brain, during both normal and under hypoxia conditions, is significantly higher than its content in the liver. Thus, in the brain under conditions of hypoxia, the content of MDA increases by 41.2 $\mu M/g$, while in the liver it increases by 30.5 $\mu M/g$, which indicates a low level of AOD in the brain and a high activity of the enzymes of this system in the liver.

Table 2

Influence of T. pratense L. and C. laevigata plants on LPO processes in tissues of rats exposed to hypoxia (n=4; p<0.01)

Experiment condition	MDA content, $\mu M/g$ tissue	
	brain	liver
Baseline	12.65 ± 1.50	6.40 ± 0.75
Hypoxia	53.85 ± 4.20	36.85 ± 3.62
Diet with *T. pratense* L. supply + hypoxia	33.15 ± 3.30	22.95 ± 2.10
Diet with *C. laevigata* supply + hypoxia	35.70 ± 3.72	14.93 ± 1.75

In the study of the antioxidant effect of plants on LPO processes, *T. pratense* L. and *C. laevigata* flowers were used, which have been added to two groups of experimental animals for 10 days, after which the animals were exposed to hypoxia. According to obtained results (Tab. 2), there is a decrease in the content of MDA under hypoxia conditions in the tissues of rats that previously received flowers of the above-mentioned plants with food.

Thus, when *T. pratense* L. flowers were added to the diet, the level of MDA in the brain decreased from 53.85 $\mu M/g$ to 33.15 $\mu M/g$, and in the liver – from 36.85 $\mu M/g$ to 22.95 $\mu M/g$. When *C. laevigata* flowers were added, the level of MDA in the brain was 35.70 $\mu M/g$, while in the liver it was 14.93 $\mu M/g$.

Thus, under the influence of *T. pratense* L. flowers, the LPO level in the brain decreases by 38.40%, and *C. laevigata* flowers – by 33.7%.

In the liver, the content of MDA decreases with the addition of *T. pratense* L. and *C. laevigata* flowers by 37.7%, and 59.5%, respectively.

It can be concluded that both plants contribute almost equally to the reduction of MDA in the brain, while in the liver the effect of *C. laevigata* flowers is more effective by 25.8% than that of *T. pratense* L. flowers. The observed slowdown of LPO processes in the liver under hypoxic conditions when using the *C. laevigata* plant indicates the activation of enzymes of the AOD system in the liver, which is apparently due to the biologically active compounds contained in the flowers.

The composition of *T. pratense* L. flowers, along with other biologically active compounds that are natural antioxidants, includes isoflavone glycosides daidzein and genistein [8, 11]. In addition to the isoflavone glycosides vitexin and acetylvitexin, the composition of *C. laevigata* flowers also contains flavonol glycosides hyperoside, quercetin, pinnatifidine, and caffeic and chlorogenic acids from other phenolic compounds [16]. A correlation was shown between the content of flavones and phenols and the antioxidant activity of plants [11].

It is no coincidence that flavones, as antioxidants, play an important role in preventing structural damage and liver functioning in various pathologies [16].

It has been shown that the following flavonols quercetin, morin and myricetin are the most active among plant antioxidants, which inhibit lipid oxidation by 78.1%, 80.6% and 83.4%, respectively [17].

Conclusion. Based on the analysis of obtained results and the literature data, we can conclude that a more effective decrease in lipid peroxidation under hypoxic conditions under the influence of *C. laevigata* compared to *T. pratense* L. is associated with its higher antioxidant activity, which is due to the content of flavonol glycosides (quercetin, hyperoside, pinnatifidine) in *C. laevigata* flowers.

Given that flavones have different effects on the AOD, it seems relevant to create combined mixtures based on medicinal plants, the use of which may enhance their pharmacological effect.

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TRIFOLIUM PRATENSE Լ. ԵՎ *CRATAEGUS LAEVIGATA* ԲՈՒՅՍԵՐԻ ՀԱԿԱՕՔՍԻԴԱՆՏԱՅԻՆ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԼԻՊԻԴՆԵՐԻ ԳԵՐՕՔՍԻԴԱՑՄԱՆ ՎՐԱ ԱՌՆԵՏՆԵՐԻ ՀՅՈՒՍՎԱԾՔՆԵՐՈՒՄ ՀԻՊՈԲԱՐԻԿ ՀԻՊՕՔՍԻԱՅԻ ՊԱՅՄԱՆՆԵՐՈՒՄ

Հետազոտվել են առնետի գլխուղեղի և լյարդի հյուսվածքներում լիպիդների գերօքսիդացման (ԼԳՕ) գործընթացները նորմալ, ինչպես նաև հիպոբարիկ հիպօքսիայի պայմաններում, քանի որ հայտնի է, որ հիպօքսիան առաջ է բերում ԼԳՕ օրգանիզմի հյուսվածքների բջիջներում: Հետազոտվել է նաև հակաօքսիդանտային ակտիվությամբ օժտված *Trifolium pratense* L.

(Երեքնուկ մարգագետնային) և *Crataegus laevigata* (Ալոճենի սովորական) բույսերի ծաղիկների ազդեցությունը հիպոբարիկ հիպօքսիայով առաջացած լիպիդների գերօքսիդացման վրա: Ցույց է տրվել, որ առնետների կերի մեջ բույսերի չորացրած ծաղիկների ավելացման դեպքում, ուղեղի և լյարդի հյուսվածքներում նվազում է ԼԳՕ-ի վերջնական արգասիքը՝ մալոնային դիալդեհիդը, առավելապես *C. Laevigata* բույսի դեպքում:

И. А. БАДАЛЯН, А. А. ПОЛАДЯН

ВЛИЯНИЕ АНТИОКСИДАНТНОЙ АКТИВНОСТИ РАСТЕНИЙ *TRIFOLIUM PRATENSE* L. И *CRATAEGUS LAEVIGATA* НА ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ В ТКАНЯХ КРЫС, ПОДВЕРГНУТЫХ ГИПОБАРИЧЕСКОЙ ГИПОКСИИ

Исследовались процессы перекисного окисления липидов (ПОЛ) в тканях головного мозга и печени крыс в нормальных условиях и в условиях гипобарической гипоксии, поскольку известно, что гипоксия вызывает развитие ПОЛ в клетках тканей организма. Исследовалось также влияние цветков растений *Trifolium pratense* L. (клевер луговой) и *Crataegus laevigata* (боярышник обыкновенный), обладающих антиоксидантной активностью, на перекисное окисление липидов, вызванное гипобарической гипоксией. Показано, что содержание конечного продукта индуцируемого гипоксией окислительного стресса – малонового диальдегида, в тканях мозга и печени крыс снижается при добавлении в рацион животных сухих цветков указанных растений, причем в случае растения *C. Laevigata* – в бóльшей степени.