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Determination of effect of *Ballóta nígra* extract on the state of lipid peroxidation and rats' antioxidant system under chronic immobilization stress

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ABSTRACT

Ability to correct free-radical peroxidation of membrane lipids in rats' organism by oral administration of Ballóta nígra extract containing complex of natural antioxidants has been investigated in experimental conditions. In experiment animals were divided on 6 groups of six animals each. Rats of group 1, which is intact (conventional condition), and rats of group 2 (exposed to the stress by immobilization for 5 hours) were intraperitoneally injected through the probe with 1.5 ml of distilled water. Animals of groups 3, 4, 5 and 6 were intraperitoneally injected through the probe with 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of Ballóta nígra extract, correspondingly, once daily prior to the stress exposition. Immobilization stress was modeled by the keeping rats for 5 hours in restraining cage. Animals of all groups were decapitated under ether anesthesia for 5 hours after modelling of immobilization stress, in other words – against the background of maximal stress exposition. Blood serum was used for investigation. Lipid peroxidation was determined, namely: level of primary oxidation products – diene conjugates (DC) and secondary products – malondialdehyde (MDA) and state of antioxidant system such as determination of catalase and superoxide dismutase (SOD) activity with a spectrophotometric method. To detect changes of parameters to be examined these levels were estimated in control and test animals after 5, 15 and 30 days, correspondingly. Taking into account the results of investigation of catalase and SOD under chronic immobilization stress we can come to conclusion that these parameters correlate better than parameters of LPO (DC and TBA-AP), this indicates on significant antioxidant and stress-modelling action of Ballóta nígra liquid extract. It has been found that Ballóta nígra extract in the dose of 1.5 and 2 ml influences on the state of LPO and antioxidant system better.

Key words: *Ballóta nígra* aqueous extract, immobilization stress, antioxidant system, lipid peroxidation.

INTRODUCTION

It is known that stress is one of the main cause of occurrence of acute and chronic diseases of different organs and body systems, particularly, neurosis, chronic fatigue syndrome, cardiovascular disease, cerebrovascular disease followed by loss of life quality [1, 2]. Progression of stress is accompanied by the abrupt increase in level of free-radical formation processes in the human body. That's why a need to use antioxidants for prophylactic and therapeutic purposes to maintain free-radical oxidation at optimal level arises [1, 3].

Treatment of nervous disorders must be complexes, focused on removal of causes of disease or stress factor that can provoke nervous system disorders.

To get positive effect at the prophylaxis and treatment of pathological states of nervous system can be achieved only by the influence on different stages of pathological process, eliminating the reason of disease and symptoms. For this purpose drug plants and phytotherapeutic medicinal products, which have sedative, neuroprotective, tonic, general tonic properties and increase specific resistance of the body, are used [4].

Ballota nigra has essential oil, bitter and tanning principles, alkaloids, pectins, organic acids, mineral substances and vitamins. *Ballota nigra* is used as sedative, antispasmodic and restorative drug. Herbal extract and powder is used at the state of nervous fever, hypochondriasis, ahypnosia, caused by nervous tension and troublesome thoughts, at stomach cramps, algomenorrhea, vasculomotor disorders [5-9].

The aim of our paper is to estimate effect of *Ballota nigra* aqueous extract on the state of lipid peroxidation and antioxidant system in rats at chronic immobilization stress.

MATERIALS AND METHODS

Neurotropic action of *Ballota nigra* aqueous extract has been studied on the model of chronic nervimuscular tension during 5, 15 and 30 days in 36 WAG rats having approximate body weight from 210 to 230 g. Immobilization stress was modeled by the keeping rats for 5 hours in restraining cage. Animals were divided on 6 groups. Rats of group 1, which is intact (conventional condition), and rats of group 2 were intraperitoneally injected through the probe with 1.5 ml of distilled water. Animals of groups 3, 4, 5 and 6 were intraperitoneally injected through the probe with 0.5 ml, 1.0 ml, 1.5 ml and 2.0 ml of *Ballóta nígra* extract, correspondingly, once daily prior to the stress exposition.

Animals of all groups were decapitated under ether anesthesia for 5 hours after modelling of immobilization stress, in other words – against the background of maximal stress exposition. Blood serum was used for investigation. Lipid peroxidation was determined, namely: level of primary oxidation products – diene conjugates (DC) and secondary products – malondialdehyde (MDA) and state of antioxidant system such as determination of catalase and superoxide dismutase (SOD) [11] activity with a spectrophotometric method [12]. To detect changes in parameters to be examined these levels were estimated in control and test animals after 5, 15 and 30 days, correspondingly.

RESULTS AND METHODS

State of POL was determined by the quantity of peroxidation products: DC and TBA-AP, which for intact animals were 14±0.64 mmol/l and 4.65±0.1 mmol/l, correspondingly. Table 1 shows that these parameters increase significantly at immobilization stress on fifth day. DC level reaches 30.72±1.06 mmol/l, that is twice as much as norm, TBA-AP reaches 6.94±0.1 mmol/l, that is half as much as control. In 15 days DC level reaches 34.85±0.85 mmol/l, that is in two and a half times greater than norm, TBA-AP reaches 7.15± 0.1 mmol/l, that is twice as much as control. In 30-thy days DC level reaches 37.85±0.12 mmol/l, that is three times greater than norm, TBA-AP reaches 7.56 ±0.78 mmol/l, that is twice as much as control.

Analysis of data from the table 1 proves that in 5 days *Ballota nigra* aqueous extract in a volume of 1.5 and 2 ml reduces level of DC and TBA-AP statistically significantly in relation to immobilization stress.

So, in immobilization stress at the dose of 1.5 and 2 ml of *Ballota nigra* aqueous extract DC level reaches 16.38±1.68 mmol/l and 15.29 ±0.65 mmol/l, correspondingly, that is significant in relation to immobilization stress 30.72±1.06 ($P<0.05$). Level of TBA-AP in immobilization stress at the dose of 1.5 and 2 ml of *Ballota nigra* aqueous extract reaches 5.25±0.17 mmol/l and 4.62±0.19 mmol/l, correspondingly, that is also statistically significant in relation to immobilization stress 6.94±0.16 mmol/l ($P<0.05$).

In 15 days *Ballota nigra* aqueous extract in all studied doses decreased DC and TBA-AP level statistically significantly in relation to immobilization stress. So, in immobilization stress at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml of *Ballota nigra* aqueous extract DC level reaches 19.12±1.11 mmol/l, 17.24±0.15 mmol/l; 15.28±1.12 mmol/l and 15.45±0.15 mmol/l, correspondingly, that is statistically significantly in relation to animal group which were subjected to immobilization stress 34.85±0.85 ($P<0.05$).

Table 1 Neurotropic and antioxidant activity of *Ballota nigra* aqueous extract

Parameter	Duration analysis	Intact animals, n = 6	IImmobilization stress, n = 6	Immobilization stress + 0.5 ml of Ballota nigra aqueous extract, n = 6	Immobilization stress + 1 ml of Ballota nigra aqueous extract, n = 6	Immobilization stress + 1. 5 ml of Ballota nigra aqueous extract, n = 6	Immobilization stress + 2 ml of Ballota nigra aqueous extract, n = 6
DC, mmol/l	5 days	14.16 ± 0.64	30.72 ± 1.06*	26.22 ± 2.11*	20.36 ± 0.22*	16.38 ± 1.68**	15.29 ± 0.65**
	15 days		34.85 ± 0.85*	19.1 2 ± 1.11**	17.24 ± 0.15**	15.28 ± 1.12 **	15.45 ± 0.15**
	30 days		37.85 ± 0.12*	17.42 ± 0.18**	16.56 ± 0.23**	14.25 ± 0.14**	14.02 ± 0 47**
MDA, µmol/l	5 days	4.65 ± 0.10	6.94 ± 0.16*	5.53 ± 0.25*	5.34 ± 0.11*	5.25 ± 0.17**	4.62 ± 0 19**
	15 days		7.15 ± 0.45*	4.31 ± 0.25**	4.23 ± 0.19**	4.05 ± 0.13**	4.34 ± 0 12**
	30 days		7.56 ± 0.78*	4.34 ± 0.12**	4.17 ± 0.15**	3.96 ± 0.17**	4.11 ± 0 43**
SOD, c.u	5 days	3.59 ± 0.11	6.93 ± 0.49*	3.23 ± 0.2**	3.14± 0.12**	2.79 ± 0.08*, **	2.3 ± 0.19*, **
	15 days		6.98 ± 0.23*	3.45 ± 0.42**	3.21 ± 0.53**	2.92 ± 0.02*, **	2.63 ± 0 12*, **
	30 days		7.13 ± 0.89*	3.26 ± 0.15**	3.15 ± 0.65**	2.45 ± 0.57*, **	2.21 ± 0 23*, **
Catalase, c.u.	5 days	5.10 ± 0.13	5.88 ± 0.26*	4.89 ± 0.12 **	4.43 ± 0.19 **	4.18 ± 0.25**	4.12 ± 0 2**
	15 days		6.03 ± 0.21*	4.21 ± 0.22 *,**	4.18 ± 0.45 *,**	4.24 ± 0.34*,**	4.34 ± 0.25*,**
	30 days		6.23 ± 0.03*	4.12 ± 0.67 *,**	3.98 ± 0.95 *,**	4.01 ± 0.17*,**	4.11 ± 0.05*,**

*Note: * - P<0.05 in comparison with the parameters of intact animals; ** - P<0.05 in comparison with the parameters of animal groups at the modelling of immobilization stress.*

Under the condition of immobilization stress at the dose of *Ballota nigra* aqueous extract 0.5 ml, 1 ml, 1.5 ml and 2 ml TBA-AP level reaches 4.31±0.25 mmol/l, 4.23±0.19 mmol/l, 4.05±0.14 mmol/l and 4.34±0.12 mmol/l correspondingly, that is statistically significant in relation to immobilization stress 6.94±0.16 mmol/l (P<0.05).

In 30 days *Ballota nigra* aqueous extract in all studied doses decreased level of DC statistically significantly in relation to immobilization stress. This level was close to control. Thus, under the condition of immobilization stress at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml of *Ballota nigra* aqueous extract DC level reaches 17.42±0.18 mmol/l, 16.56±0.23 mmol/l, 14.25±0.14 mmol/l and 14.02±0.47 mmol/l, respectively, that is statistically significantly in relation to group of animals subjected to immobilization stress 37.85±0.12 (P<0.05). Under the condition of immobilization stress at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml of *Ballota nigra* aqueous extract TBA-AP level reaches 4.34±0.212 mmol/l, 4.17±0.15 mmol/l, 3.96±0.17 nmmol/l and 4.11±0.43 mmol/l, respectively, that is statistically significantly in relation to immobilization stress 7.56±0.78 mmol/l (P<0.05).

Therefore, based on the POL level we can come to conclusion that *Ballota nigra* aqueous extract at the dose of 1.5 ml and 2 ml influences on TBA-AP level more than on DC level.

State of antioxidant system was determined by the quantity of products of catalase and SOD, which were 5.10±0.13 c.u and 3.59±0.11 c.u., respectively, in intact rats. Data given in the table 1 shows that in immobilization stress these parameters increase significantly. In 5 days, under the conditions of immobilization stress, level of catalase reaches 5.88±0.26 c.u., that is significantly over the limit (P<0.05), SOD reaches 6.93±0.49 c.u., that is twice greater than control. In 15 days level of catalase reaches 6.03±0.21 c.u. that is bigger by half than norm, but parameters of SOD reach 6.98±0.23 c.u., that is twice than control. In 30-ty days level of catalase reaches 6.23±0.03 c.u., that is twice more than norm, SOD reaches 7.13±0.89 c.u., that is in two and a half times greater than control.

Table 1 shows that in 5 days *Ballota nigra* aqueous extract influences on the level of catalase and SOD in rats' blood and statistically significant decreases these parameters in relation to immobilization stress. So, level of catalase under the conditions of immobilization stress at the dose of 0.5, 1.5 and 2 ml of *Ballota nigra* aqueous extract reaches 4.89±0.12 c.u, 4.43±0.19 c.u, 4.18±0.19 c.u and 4,.2±0.2 c.u., respectively, that is statistically significantly in relation to immobilization stress 5.88±0.26 cu. (P<0.05). Under the conditions of immobilization stress at the dose of 0.5, 1.5 and 2 ml of *Ballota nigra* aqueous extract level of SOD reaches 3.23±0.20 c.u., 3.14±0.12 c.u., 2.79±0.08 c.u. and 2.3±0.19 c.u., respectively, that is statistically significantly in relation to immobilization stress 6.93±0.15 c.u. (P<0.05).

In 15-thy days *Ballota nigra* aqueous extract in all studied doses influences on the level of catalase and SOD statistically significantly in relation to immobilization stress. This level was close to control. Thus, level of catalase under the conditions of immobilization stress at the dose of 0.5, 1.5 and 2 ml of *Ballota nigra* aqueous extract reaches 4.21±0.22 c.u., 4.18±0.45 c.u., 4.24±0.34 c.u. and 4.34±0.25 c.u., respectively, that is statistically significant in relation to group of animals subjected to immobilization stress 6.03±0.21 c.u. (P<0.05). Under the conditions of immobilization stress at the dose of 0.5, 1.5 and 2 ml of *Ballota nigra* aqueous extract level of SOD

reaches 3.45±0.42 c.u., 3.21±0.53 c.u., 2.92±0.02 c.u. and 2.63±0.12 c.u., respectively that is statistically significant in relation to immobilization stress 6.98±0.23 c.u. (P<0.05).

In 30-thy days *Ballota nigra* aqueous extract in all studied doses decreased level of catalase and SOD statistically significantly in relation to immobilization stress. Thus, under the condition of immobilization stress at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml of *Ballota nigra* aqueous extract level of catalase reaches 4.12±0.67 c.u., 3.98±0.95 c.u., 4.01±0.17 c.u. and 4.11±0.05 c.u., respectively, that is statistically significant in relation to group of animals which were subjected to immobilization stress 6.23±0.03 c.u. (P<0.05). Under the condition of immobilization stress at the dose of 0.5 ml, 1 ml, 1.5 ml and 2 ml of *Ballota nigra* aqueous extract level of SOD reaches 3.26±0.15 c.u., 3.15±0.65 c.u., 2.45±0.57 c.u. and 2.21±0.23 c.u., respectively, that is statistically significant in relation to immobilization stress 7.13±0.89 c.u. (P<0.05).

CONCLUSION

Taking into account research results of catalase and SOD under the conditions of chronic immobilizatin stress it may be concluded that these parameters are corrected better than LPO parameters (DC and TBA-AP), which is evidence of significant antioxidant and stress modelling action of *Ballóta nígra L.* liquid extract. It has been established that *Ballóta nígra* aqueous extract at the dose of 1.5 and 2 ml effects on the state of LPO and antioxidant state better.

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