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Protective effect of the *Prunella grandiflora* L. extract in relation to the toxic effect of etoposide through the example of *Drosophila melanogaster*

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ABSTRACT

The data on the protective properties of the *Prunella grandiflora L.* extract were obtained when used together with the anticancer drug etoposide on the experimental strain of *Drosophila melanogaster*. The combined use of etoposide and 10% extract of *P. grandiflora* decreased mortality in *D. melanogaster* individuals to 15% and doubled the average individual fertility compared to the use of this cytostatic drug without the extract. Using the SMART method, the presence of the antigenotoxic effect was identified, which manifests itself through the absence of chromosomal aberrations.

Key words: medicinal plants, antigenotoxic effect, *Drosophila melanogaster,* protective properties, etoposide, SMART.

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Протекторный эффект экстракта *Prunella grandiflora* L. относительно токсического воздействия этопозида на примере *Drosophila melanogaster*

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РЕЗЮМЕ

Получены данные о протекторных свойствах экстракта *Prunella grandiflora* L. (черноголовка крупноцветковая) при совместном его использовании с противораковым препаратом «Этопозид» на эксперименталь-

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ной линии животных *Drosophila melanogaster.* При совместном применении этопозида и 10%-го экстракта *P. grandiflora* показано снижение летальности у особей *D. melanogaster* до 15% и увеличение средней индивидуальной плодовитости в два раза в сравнении с использованием данного цитостатика без экстракта. Методом SMART установлено наличие антигенотоксического эффекта, который проявляется в отсутствии хромосомных аберраций.

Ключевые слова: лекарственные растения, антигенотоксический эффект, *Drosophila melanogaster,* протекторные свойства, этопозид, SMART.

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INTRODUCTION

Due to the high prevalence of cancer in the human population, the search for medicinal drugs, protectors, and adaptogens is an extremely important area of research. Cytostatics used in chemotherapy are characterized by the presence of both the main (antitumor) effect and many side effects, in particular, general toxic and genotoxic ones. To reduce side effects, various protectors are being studied, most of which are extracts from medicinal plants with a complex of various components [1].

When using an extract from medicinal plants as a protector, it is important to establish whether it itself has any toxic and genotoxic effect. In this regard, it is necessary to analyze the extract separately and together with a cytostatic drug. The use of medicinal plant extracts is widespread, a lot of such studies are carried out in India, China, and other countries where traditional medicine is common [2–4]. However, the search for protectors based on raw plant materials grown in Russia is relevant.

In this aspect, plants of the genus *Prunella* L. growing in the Ural Region are of considerable interest. The genus *Prunella* belongs to the family Lamiaceae Juss. which representatives have high biological activity and can be used to obtain valuable medicinal raw materials. *Prunella vulgaris* L. (common self-heal) is an official medicinal plant in Chinese medicine [5]. The extract from the aerial parts of *P. vulgaris* has antioxidant [6, 7], anti-inflammatory, antibacterial, antifungal [8, 9], and antitumor properties [10].

To prove these data, an article appeared in 2019 showing that *P. vulgaris* root extract inhibits *in vitro* and *in vivo* carcinogenesis in human breast carcinoma cells [11]. In Russia, *P. vulgaris* is still not a pharmacopoeial species, but in recent years it has been mentioned in the literature as a plant producing the most important classes of biologically active substances (BAS) [12–14] and has been studied as a component in pharmaceutical preparations [15]. Medicinal properties of *Prunella grandiflora* (L.) Scholler (large-flowered self-heal) are poorly studied, its extract shows antifungal and antibacterial properties and has biological activity during hypoxia [16, 17]. However, according to our assumptions, the *P. grandiflora* extract may show protective properties against anticancer drugs, as some peculiar features were revealed in the content of the main groups of BAS.

In particular, rosmarinic acid (70–89%) was found to dominate among the phenolcarboxylic acids in *P. grandiflora*. It was observed that, regardless of the harvest year, the content of rosmarinic acid was higher in *P. grandiflora* than in *P. vulgaris* [12, 18]. Rosmarinic acid has antitumor, antiproliferative [19], and anticyclooxygenase activity [20] and can protect against cancer and radiation sickness [21]. Thereafter, the aim of the study was to identify the protective properties of *P. grandiflora* in relation to the toxic and genotoxic effects of etoposide.

MATERIALS AND METHODS

The *P. grandiflora* herb was collected in the flowering phase in the Krasnoufimsk district of the Sverdlovsk region to the north of the Mariyskiy Ust-Mash village, on the Mokraya Mountain (N 56°09’22.0’’, E 058°32’19.6’’) in 2018. The plants were dried in

well-ventilated rooms. The dried raw material was ground to the size of particles passing through a 1mm sieve. A weighed portion of large-flowered self-heal in the amount of 0.8 g was extracted in 10ml of 70% alcohol for 24 hours. Then, 2.4 ml of 10% extract was added to 17.6 ml of nutrient medium. Ethyl alcohol (70%) was added to the nutrient medium in the 10 : 90 ratio, respectively, or 2.4 ml of ethyl alcohol per 17.6 ml of the nutrient medium.

We used a 20 mg / ml solution of etoposide for injections (Vero-pharm Ebave, Russia) at the concentration of 800 μg / kg of the nutrient medium. The chosen concentration of the cytostatic agent demonstrated a pronounced genotoxic effect [22]. The Oregon R laboratory line was used to assess the viability and overall mortality. For crossing, individuals were selected that were grown under the standard laboratory conditions at the temperature of 24 °C, with moderate humidity and light, in the nutrient medium containing 250 ml distilled water, 25 g glucose, 25 g yeast, and 2 g agar. For the study, the *D. melanogaster* species were used that eclosed from the puparium within no later than 6 hours (virgin females). To evaluate fertility, 25 individual pairs were placed in 25 test-tubes with a hollow lid, filled with agar medium and covered with yeast.

The laid eggs (F_1) were collected daily from the surface of the lids using a dissecting needle and placed on Petri dishes with an agar layer for further development. From the total number of eggs laid per day, the percentage of eggs that did not develop at the early stage (< 6 hours, white color) and at the late stage (> 6 hours, brown color) was calculated. To determine the mortality rate of the larvae in the parental generation of *D. melanogaster*, they were grown on the nutrient media with the extract, etoposide or the extract and etoposide combined in the amount of 300 individuals each. The overall mortality was determined by the number of individuals that died at the larval and pupal stages. At the larval stage, the difference between the number of individuals placed on the nutrient medium and the number of puparia was determined, which indicates death of unpupated larvae. Lethality at the pupal stage was identified by the presence of filled puparia in the sample, which indicates death of individuals within the puparium.

The genotoxic effect was determined using the SMART (Somatic Mutation And Recombination Test) technology. For this, females from the yellow mutant line (yellow body color, the yellow gene located on the X chromosome) were crossed with males from the white singed 3 mutant line (white eyes and singed bristles on the body, the genes are also located on the X chromosome), placing them on the test medium for 72 hours. After 72 hours, the parental generation of the flies was removed, and hybrid offspring (F_1) developed from the eggs they laid. Hybrid females of the wild phenotype (brown and gray body, straight bristles, red eyes) were used for the analysis, since the males had the yellow phenotype inherited from maternal individuals following the criss-cross phenomenon. The bristles on the female bodies were examined, and the number of bristles that were not typical of the normal phenotype in color and shape were noted, namely, yellow and / or singed ones. The area containing such bristles was recorded in the table as a single spot “y” (yellow) or “sn” (singed), or a double spot “y sn”. Statistical analysis was performed using Statistica (data analysis software system) v. 8.0., Serial Number: JPZ803I371720ARCN-6, StatSoft, Inc. When comparing and analyzing the samples, the Mann – Whitney test and the chi-square test with Yates’ correction were used.

RESULTS AND DISCUSSION

To determine the general toxic effect, two parameters were used: determination of the overall mortality of *D. melanogaster* species and their average individual fertility. The assessment of survival and mortality rates was carried out in three groups of the *D. melanogaster* species receiving the extract and etoposide separately or together during the entire period of development. After 10 days, the survival rate of the *D. melanogaster* individuals in the group grown on the nutrient medium containing the 10% *Prunella grandiflora* extract and etoposide was 72% (Fig. 1).

Figure 1 shows that the combined use of etoposide and the extract reduced the mortality rate by 20% compared to the control group and by 15% compared to the group receiving the cytostatic drug without the extract. When the *P. grandiflora* extract alone was added to the nutrient medium, no decrease in the mortality rate was found as opposed to the controls. Under the effect of 70% alcohol, the mortality rate was 36.33%. Thus, this concentration of alcohol does not have a pronounced general toxic effect and is suitable as an extract base. Consequently, the analysis of the overall mortality of individuals grown on the nutrient medium containing the 10% extract of *Prunella grandiflora L.* and etoposide showed a positive effect on their survival.

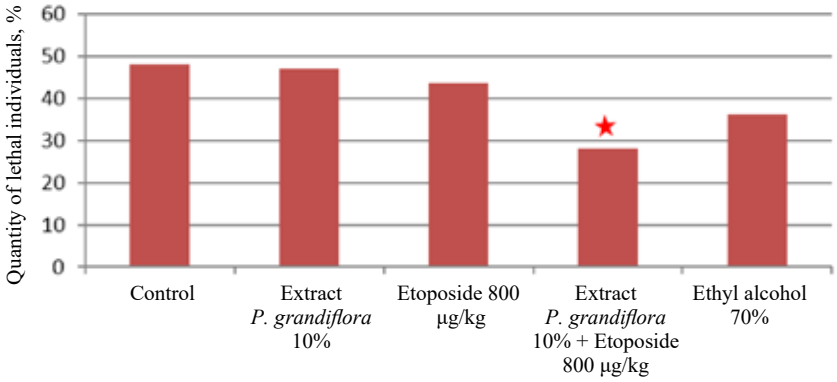

Fig. 1. The overall lethality of individuals of the Oregon-R line of *Drosophila melanogaster*, grown on the nutrient medium with addition of various components: * values that significantly differ from the corresponding parameters in the control group, $p < 0.001$ (chi-square test)

The parameters of viability of the Oregon-R line were assessed, such as the average individual fertility (AIF), and the frequency of early and late lethality of the offspring at the embryonic stage (up to 6 hours of development – early embryonic lethality (EEL), after – late embryonic lethality (LEL)). Figure 2 demonstrates the improvement in fertility rates when etoposide and the extract were used together and when the extract was used alone.

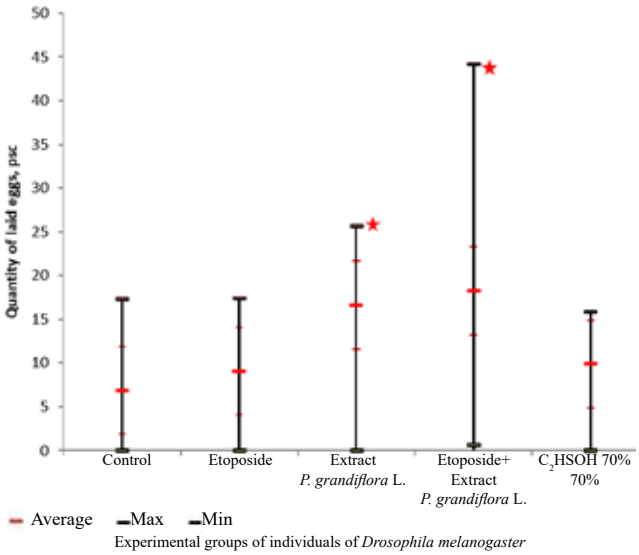

Fig. 2. Average individual fertility of the Oregon-R line of *Drosophila melanogaster* grown on the nutrient medium with addition of various tested substances: * values that significantly differ from the corresponding parameters in the control group, $p < 0.05$ (Mann – Whitney test)

A decrease in the general toxic effect was registered under the effect of the extract, which manifested itself through a change in the AIF indicator: when receiving etoposide, the AIF was 9.03; when the cytostatic and the extract were combined, it went up to 18.27. In addition, the comparative characteristics of AIF in the studied flies showed that the maximum number of laid eggs was recorded when the extract was used with etoposide. Therefore, the extract neutralizes the toxic effect on fertility potential. However, such pronounced effect does not apply to the mortality rate of the offspring at the embryonic stage. Perhaps this is explained by the short duration of the positive effect of the extract for changing the lethality index F_1. It should be noted that the results of AIF obtained using 70% alcohol, which is the base of the extract, are comparable to those obtained using etoposide.

The genotoxic effect of etoposide was also analyzed using SMART lines. An increase in the frequency of mutations and recombinations with a rise in the concentration in the nutrient medium was observed (Table). Table 1 shows that the extract itself does not change the frequency of mutations and recombinations, thus, it does not show genotoxic properties, while etoposide has a clear genotoxic effect, which complies with the results of other studies [23].

The genotoxicity of 70% alcohol manifests itself in the form of single singed spots, and therefore, it can be assumed that its action specifically damages the chromosome in the periventricular region. The absence of females with yellow spots allows to suppose that the eliminated chromosome region was not exposed to the active effect of alcohol. At the same time, the chi-square value in the experimental group receiving etoposide 400 µg / kg, as opposed to the controls, was close to the critical one.

Table 1

Characteristics of somatic mosaicism in *Drosophila melanogaster* using yellow (y) and singed (sn) markers

Test groups	Sample	Number of individuals with mutant spots					(χ2)	(*p*)
		y	sn	y sn	Other mutant phenotypes	Sample proportion, %		
Control	573	1	2	0	0	0.52	–	–
Etoposide (400 μg / kg)	499	0	11	0	0	2.2	4.615	0.032
Etoposide (800 μg / kg)	196	0	0	0	9	4.59	13.199	‹0.001
P. grandiflora extract, 10%	176	0	2	0	0	1.14	0.118	0.731
Alcohol, 70%	227	0	7	0	0	3.08	6.68	0.010
P. grandiflora extract, 10% + etoposide (800 μg / kg)	230	1	5	1	0	3.04	6.55	‹0.011

In addition, when using etoposide at the concentration of 800 μg / kg of nutrient medium, a large number of non-characteristic mutant recessive phenotypes was recorded that resulted from the pseudodominance phenomenon, which indirectly demonstrates the effect of this cytostatic agent on the frequency of chromosomal aberrations. According to A.N. Sortibran et al., an increase in chromosomal rearrangements does indeed take place [24].

When the extract and etoposide were used together, no change was found in the genotoxic properties of etoposide in relation to the frequency of spot occurrence, however, the antigenotoxic effect was recorded in relation to the frequency of chromosomal aberrations. Therefore, it can be asserted that the extract is selective for the genotoxic properties of etoposide. Since extracts of other medicinal plants were used both in high and low concentrations for the manifestation of antigenotoxic properties [25], it seems reasonable to test this hypothesis with respect to the *P. grandiflora* extract used in this study.

CONCLUSION

The discovery of a positive effect of the 10% *P. grandiflora* extract on antigenotoxicity, overall lethality, and viability of the Oregon-R *D. melanogaster* individuals exposed to etoposide and the extract makes further testing of this extract reasonable. The data obtained allow to consider the possibility of using the *P. grandiflora* extract as a component in the diet of patients undergoing certain therapeutic treatment.

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