Biologically active properties of *Betonica officinalis* L. growing in the Middle Urals of Russia

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Abstract

Testing the biologically active properties of medicinal plants is an integral part of their rational use. In this work, we determined the effect of ethanol extract of *Betonica officinalis* L. on the viability of *Drosophila* as a model object. A decrease in fertility and an increase in the lethality of offspring of first generation at the embryonic development stage were revealed. No change in the lethality of adults grown on the medium with the addition of the extract was recorded, although the peak of lethality shifted with an increase in concentration. Ovarian atrophy was not observed when the larvae were grown on medium with extract of *Betonica officinalis* L.

Keywords: Extract, *Drosophila*, Stress, lethality

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Introduction

Natural medicinal raw materials have several advantages over synthetic drugs used or intended for use in medical practice. The complex of biologically active substances underlying plant extracts is less aggressive towards genetic material, tissues and organs. Representatives of the Lamiaceae family have established themselves as a source of broad-spectrum agents in pharmacognosy. Stachys and Salvia have established themselves as containing species with biologically active components, the biochemical composition of which makes up their potential as medicinal raw materials.

Medicinal plants are a significant source of phytochemical substances that are essential for maintaining human health (Gurusamy et al., 2022). It is a wealth of phytochemicals that have antigenotoxic, adaptogenic anti-urolithiasis, anti-tumor, antioxidant, antiviral, antibacterial, and antifungal qualities, which makes them useful for drug discovery and wound healing (Dhanaraj et al., 2025; Gurusamy et al., 2024; Raj et al., 2024; Manikandan et al., 2022; Manikandan et al., 2021; Manikandan et al., 2020; Surendran et al., 2020; Manikandan et al., 2019; Manikandan et al., 2017). In drug discovery, these plants' phytochemicals are crucial for producing pharmaceutical product analogs (Packiyalakshmi et al., 2017; Manikandan & Ramasubbu, 2020; Manikandan & Ramasubbu, 2023; Paniagua-Zambrana and Bussmann 2024). *Betonica officinalis* L. is an under investigated member of the Lamiaceae family, which includes a wide variety of medicinal plants. According to research, *B. officinalis* is characterized by a high content of chlorogenic and caffeic acids, and is rich in polyphenols and tannins (Paun et al. 2016). A similar biochemical basis of MPRM ensures the manifestation of antidiabetic potential in the form of inhibition of alpha-amylase and alpha-glucosidase (Paun et al. 2016).

The few studies of the extract indicate that *Betonica* has some genotoxic potential, as the authors found an increase in SCE (sister chromatid exchange) and DNA damage (Slapšytė et al. 2019). However, the Ames test for this extract did not reveal genetic activity.

The pronounced anti-influenza activity of the ethanol extract of *B. officinalis* has also been described in relation to the neutralization of the H3N2 subtype of influenza A virus (Protsenko et al. 2021). According to the authors, these properties are ensured due to the high content of catechins in this MPRM.

It has been shown that the content of tannins depends on growing conditions and determines the ability of *B. officinalis* to adapt to the conditions of the forest-steppe or taiga zones (Morilov et al. 2020). According to previous studies, it can be assumed that *B. officinalis* extracts have antitumor activity. The search for a new type of raw material that has an antitumor effect without side effects is a current line of biomedical research.

The use of *Drosophila melanogaster* as a model object for testing the biologically active properties of medicinal plants has a number of advantages: a short 10-day life cycle, economical use and cultivation of individuals, the presence of a large number of specific lines and good coverage of studies. And the main important criterion for the experimental application of this model in vivo is the high gene homology between *D. melanogaster* and mammals (Ben-Zur 2000; MacDonald et al. 2010).

In this connection, the purpose of this study was to determine the biologically active properties of the ethanol extract of *Betonica officinalis* L., growing in the forest-steppe conditions of the Ural region of Russia.

Materials and Methods

Collection of medicinal plant materials

The collection of *Betonica officinalis* was carried out in the Mechetlinsky district of the Republic of Bashkortostan near the village of Kurgatovo; 1,2 km to the north, Mount «Gnezdo Berkuta» (56*09"22.0""N 58*32"19,6"), Voucher number UFU – T09020.

Extraction of Plant materials

Drying was carried out in a well-ventilated area. Grinding was carried out to a particle size of no more than 1 mm. The ethanol extract was prepared in a ratio of 1:10 dry above-ground part of medicinal plant and extractant (70% ethanol).

Drosophila lines

The Oregon-R line (an isogenic wild-type laboratory line) of *Drosophila melanogaster* was used in this work. The line is from the collection of Department Biodiversity and Bioecology of Ural Federal University.

Determination of lethality, adaptogenicity and life expectancy

The extract of *B. officinalis* was added to the nutrient medium for cultivating *Drosophila*. The extract was tested for lethality by adding from 1% to 10% relative to the volume of the nutrient medium.

Adaptogenicity was determined in individuals grown on a nutrient medium supplemented with 2% (40 mg/ml) and 5% (100 mg/ml) of the extract relative to the total volume of the medium. Females and males separately in the amount of 50 individuals were placed under the conditions of hyperthermic stress in a thermostat of 35 °C. The number of dead adults was recorded every 24 hours.

Lifespan was carried out on a minimal medium (agar 2g, water 250g, glucose 250g) in vials of 100 individuals of each sex, kept separately. Dead flies were recorded and vials were changed every 3 days.

Fertility and embryonic lethality of F_1 offspring were determined in individuals grown on tested nutrient media with the addition of the extract. 25 vials with individual female-male pairs in each experiment were tested for 10 days in relation to fertile potential: laid eggs were collected, among which, after the completion of embryonic development, undeveloped eggs which were dechorionized were counted and the stage of embryonic development at which the death of the offspring F_1 occurred was determined.

Ovarian atrophy was determined in females of the experimental groups by dissecting the gonads in phosphate-buffered saline, after which both ovaries were fixed in a drop of glycerine and their morphological features were determined: size, development and completeness.

Statistical data processing

Statistical data processing was carried out using the Statistica 13.0, GraphPad Prism 6.0 program. The chi-square test was used to assess mortality and ovarian atrophy, and the t-test was used to assess fertility and embryonic lethality. Life expectancy under normal conditions and under hyperthermia conditions was assessed using the Log-rank (Mantel-Cox) test. The work took into account a significant difference at $p<0.05$.

Results and Discussion

Result

The extract of *Betonica officinalis* was added to the nutrient medium at concentrations of 1%, 2%, 3%, 4%, 5% and 10% of the total volume of the substrate. In all experimental groups no toxic or antitoxic manifestations were recorded (Figure 1). The lowest mortality rate in the range of variation was recorded for 2% of the experimental group among those tested, and therefore it was chosen for further work, also using the 5% option for comparison.

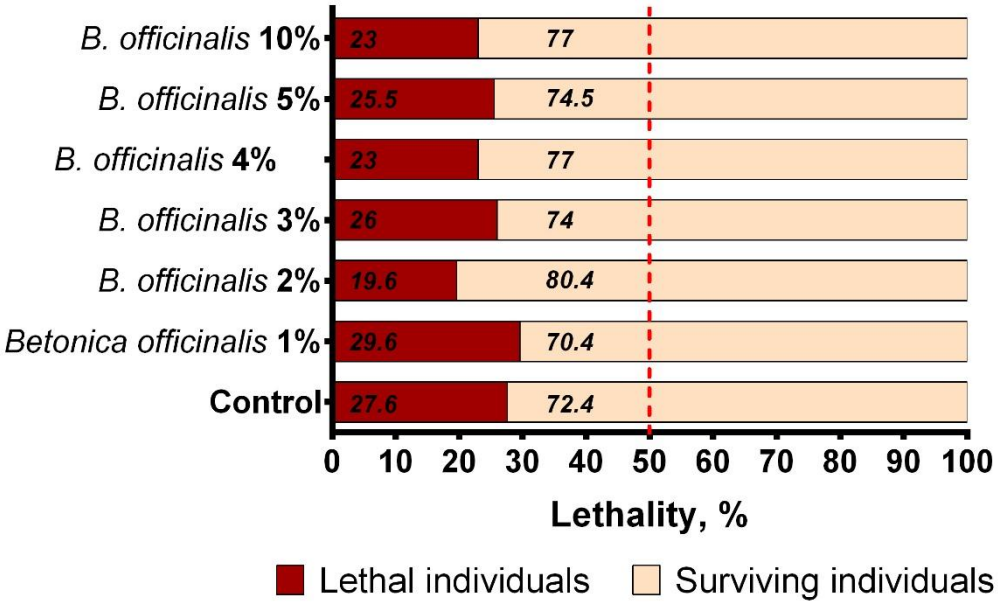

Figure 1: Survival rate of *D. melanogaster* individuals when grown on experimental nutrient media supplemented with *B. officinalis* extract.

When analyzing adaptogenicity to hyperthermic stress (35 °C), significant differences were revealed in the mortality curves of females ($\chi2 = 41.89$, $p<0.0001$) (Figure 2A) and males ($\chi2 = 59.7$, $p<0.0001$) (Figure 2B). The median death of females in the control group

occurs at 72nd hour of hyperthermia, while in the experimental groups of 2% and 5% extracts this figure is 48 hours and, compared with the control, the mortality curves have a significant difference (2%: $\chi 2 = 28.72$, $p<0.0001$ и 5%: $\chi 2 = 17.23$, $p<0.0001$).

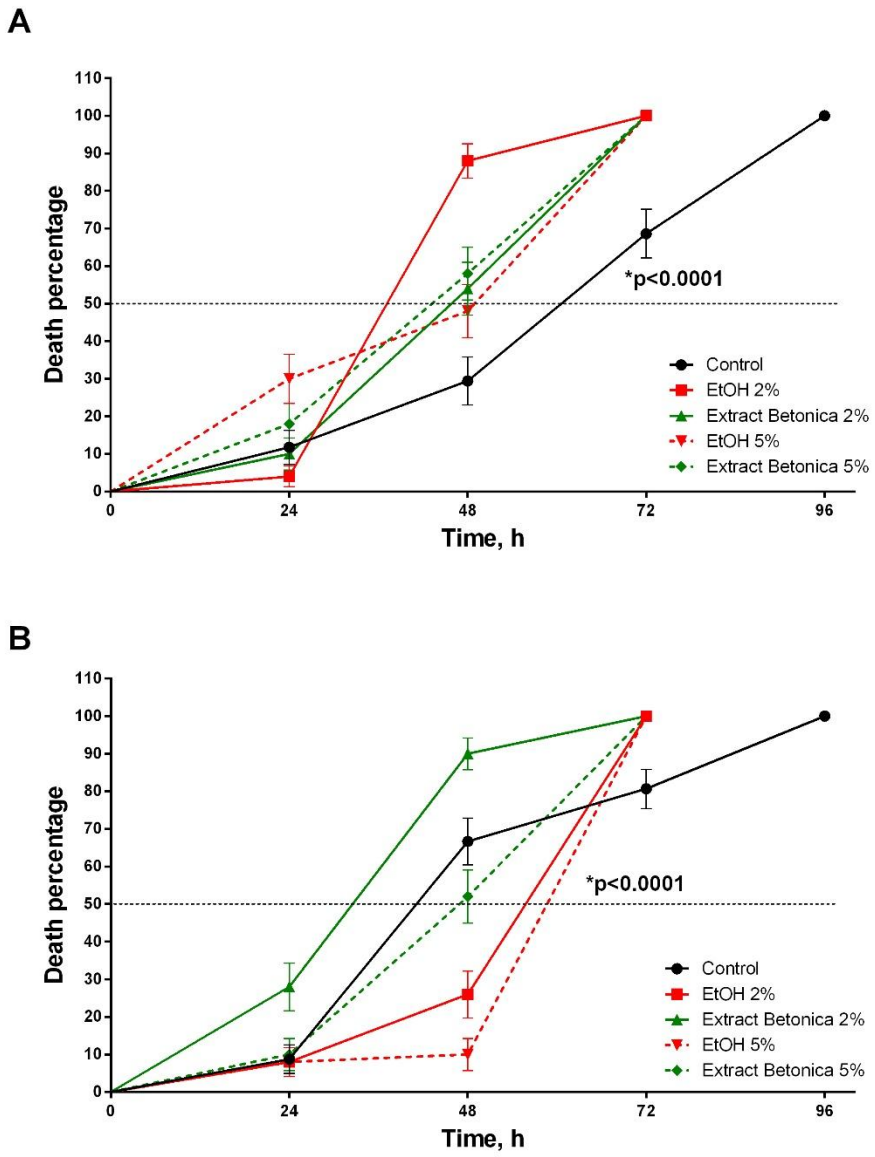

Figure 2: Effect of B. *officinalis* extract on mortality dynamics of *D. melanogaster* females (A) and males (B) under conditions of hyperthermic stress.

For males, a pronounced effect of hyperthermia on survival was also revealed. However, the mortality curves of control males differ from those of females. It was shown that the median death of males in the control group was 48 hours.

We also tested the lifespan in non-stress conditions of females and males of the experimental groups (Figures 3A and 3B). Significant differences were obtained in the survival curves of

females ($\chi 2$ = 26.17, $p<0.0001$) and males ($\chi 2$ = 26.02, $p<0.0001$). It was found that the median lifespan for females in the control group was 27 days, and for groups of females receiving 2% and 5% ethanol extracts of *B. officinalis*, this figure was on 30th and 36th day. At the same time, a significant difference was detected only for 5% of the extract ($\chi 2$ = 31.75, $p<0.0001$).

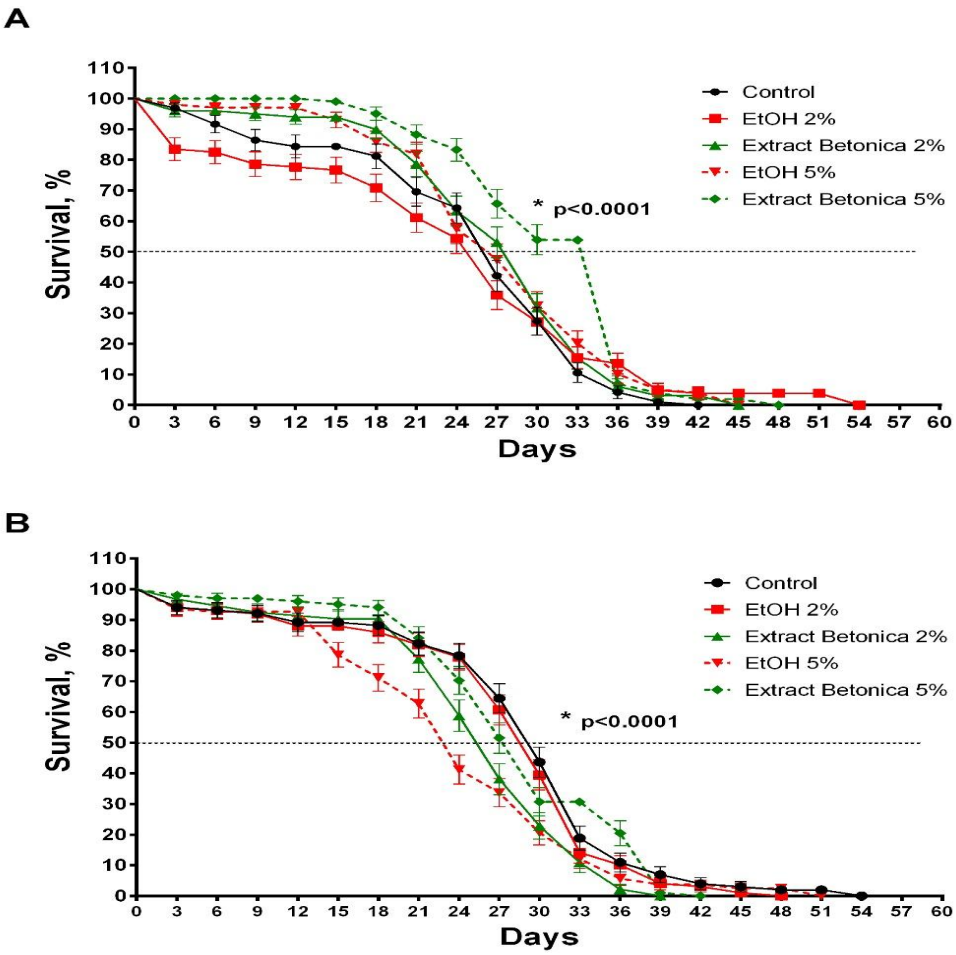

Figure 3: Effect of *B. officinalis* extract on the lifespan of *D. melanogaster* females (A) and males (B).

In the group of males receiving 2% and 5% ethanol extracts of *B. officinalis*, we did not detect a protective effect neither in terms of the median lifespan nor in the 90% death rate of the sample.

The fertility of individuals from different experimental groups was analyzed over a 10-day period. In both experimental groups grown on a medium with the addition of the extract, a decrease in fertility was observed relative to the control sample (Fig. 4). In the same experimental groups, an increase in embryonic lethality of offspring was recorded in the early

stages of development (up to 6 hours) (Fig. 5). Whereas with regard to embryonic offspring lethality at later stages of development (after 6 hours), no increase in mortality was recorded.

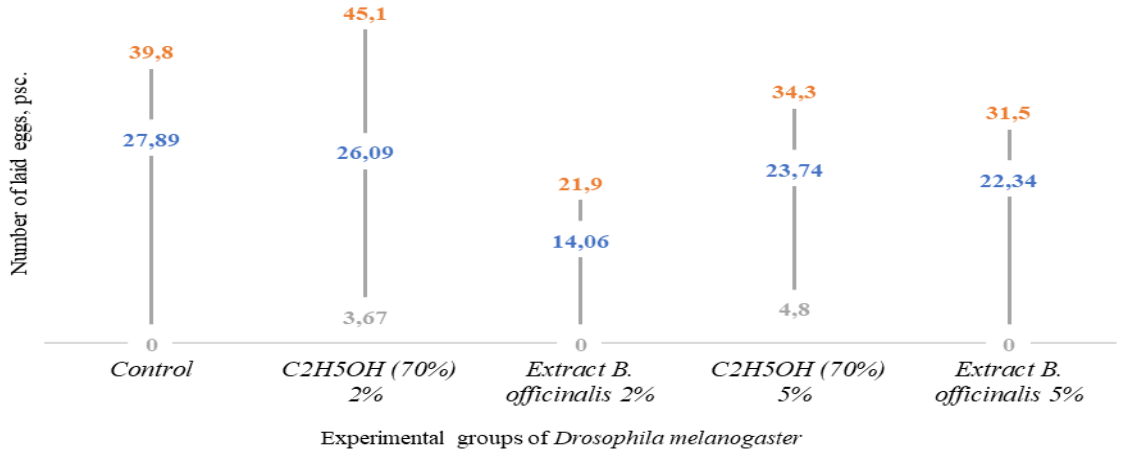

Figure 4: Average individual fecundity of individuals of different experimental groups of *D. melanogaster*.

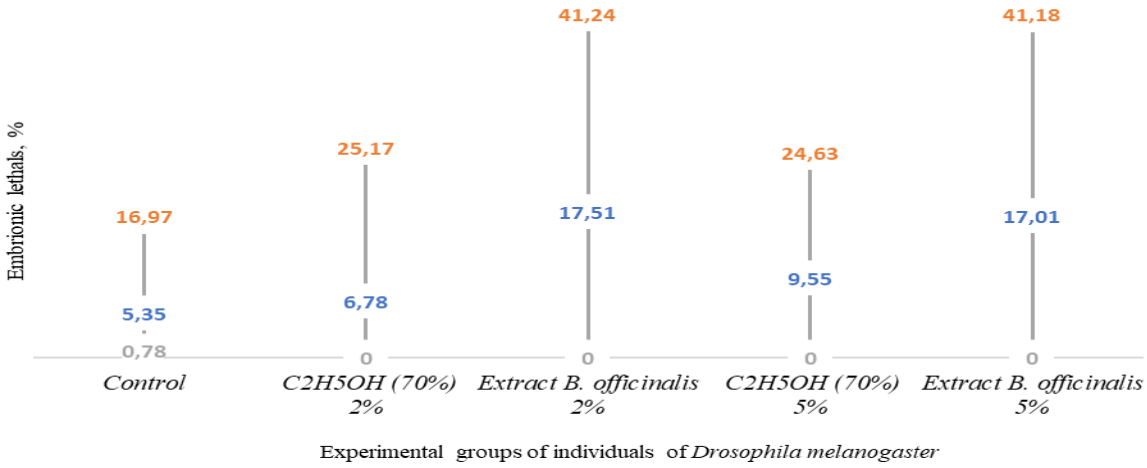

Figure 5: Early embryonic lethality of F1 offspring of different experimental groups of *D. melanogaster*.

Due to decreased fertility, ovarian atrophy of adult females from all experimental test groups was analyzed to identify the cause. When the extract was used in both 2% and 5% concentrations, the degree of atrophy did not increase, which indicates that the decrease in fertility is not associated with a violation of the structure and normal development of ovarioles in the ovaries. In the group of females raised on a medium with the addition of the extract at a concentration of 2% relative to the total volume of the nutrient substrate, a single

case of atrophy of one of the ovaries was found ($\chi 2 = 0,63$; $p = 0,43$), as well as cases of gonadal disproportion ($\chi 2 = 0,03$; $p = 0,87$), but such fluctuations are within the control sample (Fig. 6).

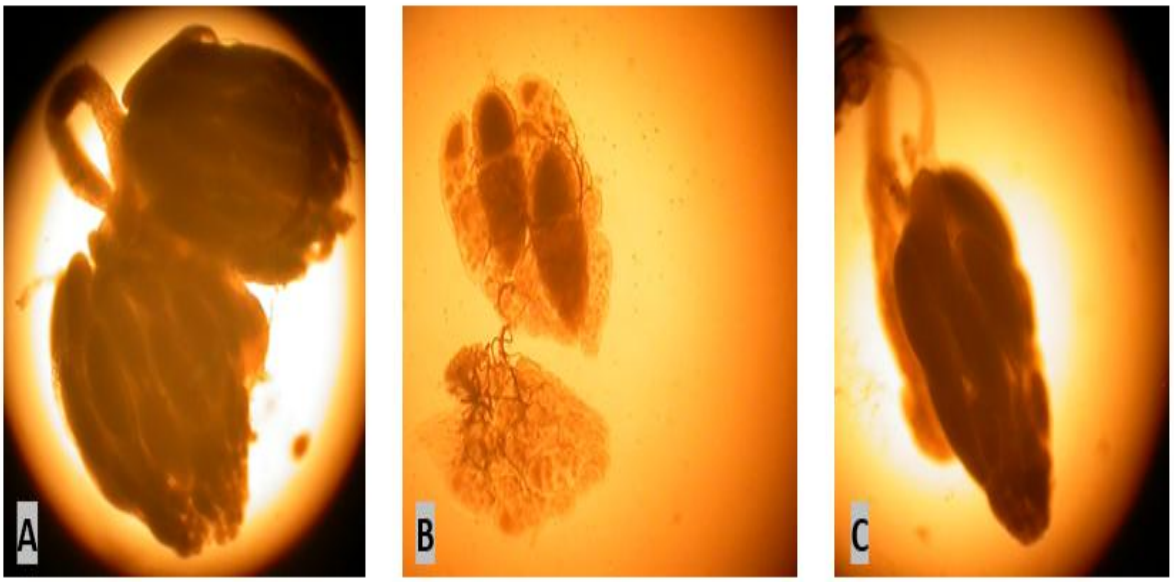

Figure 6: Ovaries of female *D. melanogaster* grown on a medium supplemented with 2% *B. officinalis* extract (A – Proportionate normally developed ovaries, B – Disproportionate normally developed ovaries, C – Disproportionate ovaries, 1 atrophied).

Having diagnosed an increase in the mortality rate of F1 offspring at the early embryonic stage in groups of individuals grown on a medium with the addition of the extract, we identified marker stages of embryogenesis up to the 6th hour of development. It was found that most often the offspring of individuals cultured on a medium with a 2% extract stops developing at stages 5-6 of early embryogenesis (Fig. 7 A-B).

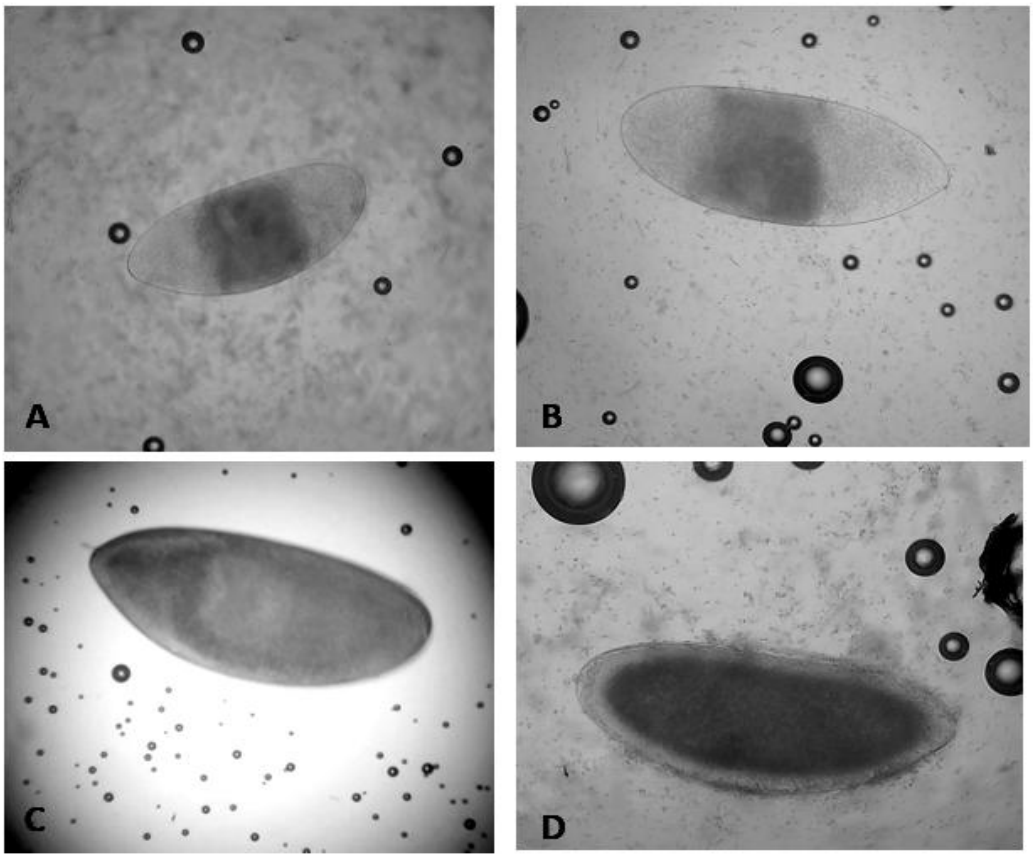

Figure 7: Stages of disruption of early embryogenesis of *Drosophila* F1 when adults are fed the substrate with the addition of *B. officinalis* extract 2% (A-B – stages 5-6 of synchronous central nuclear division, C – stage of formation of the cephalic groove, D – stage of cellular blastoderm).

Later arrest of early development is less common and is more often represented by the stages of formation of the cephalic groove and cellular blastoderm (Fig. 7 C-D). Accordingly, development often stops at very early stages of embryogenesis, namely 1,5-3 hours.

Discussion

The registered increase in embryonic lethality of offspring at the stage of early embryogenesis in individuals cultivated on a medium supplemented with ethanol extract of *Betonica officinalis* may indicate the presence of genetic activity. Active damage to DNA, chromosome structure and activation of mobile genetic elements can lead to very early disruption of the functioning of morphogenesis genes, including zygotic genes, which is

reflected in an increased incidence of mortality (Kuntz and Eisen 2014). According to the authors (Paun et al. 2016), the *B. officinalis* extract exhibits genotoxic properties. In turn, with an increase in the concentration of the extract, the peak of mortality in adulthood shifts, although the life expectancy itself remains within the control sample. In this connection, lethality during embryogenesis is not associated with the lethality of adults, which also supports the assumption that the genetic activity of the extract has a more pronounced effect in the early stages of ontogenesis (Morilov 2014).

The detected decrease in fertility is often observed as an accompanying process of response to stress, in the presence of a certain complex of biologically active compounds in the *B. officinalis* herb. According to Morilov's data, the 2014 modification complex of *B. officinalis* of a forb meadow in the forest-steppe zone of the Krasnoufimsky district of the Sverdlovsk region of Russia is characterized by shorter generative shoots and small upper stem leaves, which correlates with an increased content of tannins and has adaptive significance for plants in this zone (Tarasova and Batlutskaya 2013). In the taiga zone, the composition of the *B. officinalis* essential oil is represented by sesquiterpenoids with a predominance of β-caryophyllene (25,9%) and germacrene D (32,7%) (Novakovskaya and Punegov 2010). An excess of such compounds can have negative consequences, for example, in the form of cell cycle disruption (Isaenko and Shvartsman 1999). Such hormone as 20-hydroxyecdysone is an important marker for the functioning of the ovaries, and its destabilization that occurs during nutritional stress can lead to decreased fertility and increased mortality of offspring at both the embryonic and postembryonic stages (Bobrovskikh and Gruntenko 2023). In this connection, the effect observed with the action of the ethanol extract of *B. officinalis* may be the result of hormonal changes in individuals grown on experimental nutrient media. Also, atrophy (complete or partial) of the gonads observed in our experiment may be the result of genetic instability of the genome (activation of MGE) during the development of the insect genital imaginal discs and affect the reproductive ability of the adults (Yushkova 2017). One of the possible mechanisms of the negative effect of *Betonica* on fertility and fecundity, as well as the survival of Drosophila, may lie in the natural herbicidal/insecticidal properties of the plant, manifested through a violation of the integrity of the mitochondrial membrane, ATP deficiency, and as a consequence, the direction of the cell towards apoptosis (Kanthasamy et al. 2019). In addition, *Betonica* toxic compounds can interfere with microtubule assembly, form dimers with tubullin, arrest mitosis and inhibit cell proliferation.

Conclusion

The *B. officinalis* extract is characterized by the absence of a toxic effect at the stage of postembryonic development and adult, as well as the absence of changes in gerontological parameters. At the same time, the extract affects the fertile potential and viability of F_1 offspring. These effects are not associated with the process of gonadal atrophy; perhaps they are realized due to the genetic activity of the extract and the active influence on the expression of genes of early embryogenesis.

Author contributions

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A.D. Mokrousov: student, Ural Federal University, Yekaterinburg, Russia, no ORCID, the experimental part of the work on the identification of stages of embryogenesis and ovarian atrophy.

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