Effect of *Heracleum sosnowskyi* extract aqueous solution on the *Allium cepa* root meristem

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**Abstract**

*Heracleum sosnowskyi* (Apiaceae) contains a lot of useful chemical ingredients that can be used in industry, medicine and other fields as plant component extracts and as chemical compounds that have been extracted in different ways, which requires the last to be tested for chemical safety, including a genotoxic test in vivo. In the present paper, the 96-hour effect of the *H. sosnowskyi* extract aqueous solution at concentrations of 0.01, 0.05, 0.10, and 0.50 mL/L on the genetic apparatus and mitotic activity of the cells of the *Allium cepa* (Alliaceae) root meristem is discussed. Distilled water was applied as a negative control, and hydrogen peroxide 1% as a positive one. The extract was prepared from the plant’s fresh leaves by soaking them in acetone. It was then distilled at 57 ºC and diluted with distilled water to obtain the experimental concentrations. As extract content in the aqueous solution increased, a statistically significant decrease in mitotic activity, an increase in aberrant cell percentage and a concentration-dependent inhibition of root growth were observed. In the 0.5 mL/L solution, if compared against the other experimental concentrations, an increase in the metaphase, anaphase and telophase indices along with a decrease in the prophase index were observed. The most common aberrations for all the concentrations were lagging and sticking chromosomes, anaphase bridges, ring chromosomes and nuclear buds. The same solution and the positive control produced membrane damage; giant and ghost cells. The results of the experiment performed have demonstrated the extract’s aneugenic effect that causes spindle disturbance, mitodepression and inhibits the cells of the *Allium cepa* root meristem, prevails over its clastogenic effect.

**Keywords**

Genotoxicity; mitosis; plant extract; mitotic activity; lagging and sticking chromosomes; anaphase bridges; ring chromosomes; nuclear buds.

**Introduction**

In Russia and many other countries, *Heracleum sosnowskyi* has ceased to be a promising forage plant and turned into an invasive species (Tkachenko, 2015; Visosciene, 2020). Excrete allopatic substrates and spreading a huge number of seeds (Jukubskas-Busse, 2013), the plant conquers new territories and present a clear danger for the biodiversity, economies and the people of the land. Its juice has photosensitizing action making it potentially hazardous for humans. Some data say the juice of other hogweed species cause mutations in mammal lymphocytes (Bobuaka-Kooch et al., 2008) and is toxic for aquatic organisms even without photoactivation (Moshaif et al., 2009).

Although the hogweed that has failed to become a proper greening plant and livestock feed, is a great raw material available in large quantities. For instance, the aboveground part of *H. sosnowskyi* is currently tested as an additive for a construction material (Musorina et al., 2019) and can be used in cardboard production (Musikhin & Sigaev, 2006). As for its chemical composition, the plant is rich in proteins, vitamins, furanocoumarins, ethers, etc. making it a proper raw material for manufacturing food additives and in this way reducing its harmfulness and rendering it potentially useful in medicine and science (Jahodová et al., 2007; Shakhmatov et al., 2013). The plan most commonly used for cytological and genotoxic monitoring has long been *Allium cepa* due to the ease of working with the cells of its root meristem that are large (both cells and chromosomes) and of small quantity (2n = 16). The *Allium* assay has been the standard sensitivity test in vivo and its results can be used to assess genotoxicity for other living forms, including humans (Fiskesjo, 1985; Tedesco & Laughinghouse, 2012; Bonciu et al., 2018). In the presented study, the *Allium* assay was used to estimate the geno-and cytotoxicity of the *H. sosnowskyi* extract aqueous solution.

**Materials and methods**

The leaves of *H. sosnowskyi* collected from a boggy roadside (67.601986°N 33.416213°E) were soaked in acetone in proportion 1:1 (1 kg of leaves). The solution was distilled at 57 ºC. The residue, a grained dark-green liquid, was diluted with distilled water to obtain an aqueous solution at concentrations of 0.01, 0.05, 0.10, and 0.50 mL/L.

The bulbs *Allium cepa* of Stuttgarter Riesen (2n = 16) bought in a shop were kept in a dark, cold room for 14 days. From these, bulbs of similar diameter were selected and their dead-skin layer was removed. Following the test design by Fiskesjo (Wang et al., 1997), the selected...
bulbs were left to germinate in distilled water for 24 hours. After the germination period, 40 bulbs were selected (5 bulbs per concentration). As controls, bulbs with 2–3 mm roots were selected. Distilled water was applied as a negative control, and hydrogen peroxide 1% as a positive one (Akou et al., 2019). The experiments lasted for 96 hours in a dark room at room temperature. All the exposure conditions and monitor data were encrypted. Upon completion, the roots were cut off; their length measured in millimeters to estimate their growth (Fiskejo, 1985; Wierzbicka & Antosiewicz, 1988; O’Hare et al., 1995). The roots were fixed in vinegar alcohol (96% of ethanol + glacial acetic acid in proportion 3:1) for a day and then rinsed three times in 80% ethanol (one hour each time) to be placed in sealable test-tubes for long-time storing.

To prepare the specimen, the roots were hydrolyzed and stained in ceramic crucibles in boiling 2% aceto-orcein stain solution (NPP PanEco, Russia). After cooling, the crucibles were left for 24–72 hours at 4 ºC for the roots to stain (Medvedeva et al., 2014).

For every mentioned concentration and control, 15 squash specimen samples were prepared from 15 roots. A root tip with growth zone of 3–4 mm in length was cut off with a scalpel to be placed on a specimen glass in a drop of glacial acetic acid. Then it was covered by a cover glass, pressed with a napkin and accurately squeezed by the tapping motions of the blunt tip of a glass rod. The edges of the cover glass were then sealed with nail polish to prevent the acetic acid evaporating and extend the observation time. For each specimen, about 1000 cells were counted with phase and chromosome aberration marked at ×400 and ×1000 (in immersion oil) magnification of a Microman 1, v. 1–20 microscope (Microman, Russia, 2019). The shooting was performed using a digital Toupcam 2.0 Cmos Camera (Toupiec, China, 2019) equipped with the TouView software for the 1/2.7" sensor of 1920 x 1080 pixel resolution. In total, more than 90000 cells were counted. The mitotic index (MI) was calculated as a percentage ratio of the number of all dividing cells to the general number of the cells calculated in a specimen. The proportions of mitotic phases were calculated in the same way.

Statistical analysis was carried out using the R programming language (free software environment for statistical computing, New Zealand). The sample distribution normality was determined following the Shapiro-Wilk test. The differences in MI; root length; between experiment and control groups were verified with a one-way ANOVA. The level of significance was accepted to be P < 0.05. Additionally, the Tukey multiple pair-wise comparisons were performed. The differences in phase indices were calculated using the Kruskal-Wallis statistic and Dunn’s multiple comparison post hoc test.

Results

The *H. sosnowskyi* aqueous solution had an antiproliferative effect on the cells of the *A. cepa* root as can be seen from the results of graphical and statistical analysis of root lengths and mitotic index (Fig. 1).

| Concentration, mL/L | MI, % | NC | 0.01 | 0.05 | 0.10 | 0.50 | PC |
|---------------------|------|----|------|------|------|------|----|
| Control (distilled water) | 52.7 ± 8.2 | 82.3 ± 6.4 | 83.2 ± 2.8 | 3.2 ± 1.2 | 6.2 ± 3.5 |
| 0.01 | 42.6 ± 7.9 | 76.7 ± 9.8 | 76.1 ± 5.4 | 3.6 ± 2.1 | 7.5 ± 3.0 |
| 0.05 | 42.3 ± 6.5 | 82.5 ± 3.7 | 6.2 ± 2.5 | 5.0 ± 2.0 | 4.9 ± 1.7 |
| 0.10 | 24.3 ± 6.4 | 75.5 ± 12.9 | 11.6 ± 6.8 | 5.2 ± 3.0 | 7.8 ± 4.2 |
| 0.50 | 12.6 ± 4.2 | 23.1 ± 11.5 | 33.9 ± 9.4 | 21.2 ± 8.6 | 21.9 ± 7.1 |
| Positive control (H₂O₂, 1%) | 3.9 ± 2.2 | 88.1 ± 25.7 | 5.9 ± 15.9 | 4.7 ± 9.9 | 1.3 ± 2.0 |

Note: different letters indicate values that differ significantly from each other in the same columns of the table by comparison with the Dunn’s test P < 0.05.
additive and determine the changes of genetic material. To register and estimate these changes different test systems are applied, higher plants among them.

Table 2
Aberrations in the dividing cells of the *A. cepa* roots treated with the *H. sosnowskyi* extract aqueous solution

| Concentration, mL/L | Chromosome lagging, cells | Chromosome stickiness, cells | Anaphase bridges, cells | Ring chromosomes, cells | Aberrant mitotic cells*, % |
|---------------------|--------------------------|-----------------------------|------------------------|------------------------|--------------------------|
| NC, H2O distilled    | 8                        | 0                           | 3                      | 0                      | 0.2                      |
| PC, H2O2, 1%         | 1                        | 36                          | 0                      | 6.3                    |
| 0.50                | 89                       | 88                          | 10                     | 9.9                    |
| 0.10                | 107                      | 53                          | 5                      | 4.5                    |
| 0.05                | 50                       | 58                          | 9                      | 1.8                    |
| 0.01                | 22                       | 47                          | 0                      | 1.3                    |

Note: the calculations were made in relation to the number of dividing cells.

The following aberrations formed a separated group: giant cells; enucleate (ghost) cells and cells with defective interphase membranes (Table 3). These anomalies occurred either in the solution with the highest extract concentration or in H2O2, 1% (Fig. 5).

Discussion

*H. sosnowskyi* is currently considered as a source of biologically active compounds such as furcoumarins, e.g. its leaves contain angelicin, bergapten, methoxsalen and umbelliferon. Bergapten and methoxsalen are used to treat certain skin diseases, and umbelliferon has anticoagulation properties and is applied for thrombosis treatment (Georgievsky et al., 1990). In our study hogweed extract aqueous solution was investigated, which, as the aqueous solutions of other plant extracts, is a complex mixture of chemical compounds that have their effect on the processes occurring in a living organism. These effects can be synergistic, antagonistic or

Table 3
Aberrations in the meristematic cells of the *A. cepa* roots treated with the *H. sosnowskyi* extract aqueous solution (N = 15000)

| Concentration, mL/L | Nuclear buds, cells | Giant cells | Cells with defective membranes | Aberrant cells*, % |
|---------------------|--------------------|-------------|-------------------------------|-------------------|
| PC, H2O2, 1%        | 0                  | 89          | 125                           | 1.4               |
| 0.5                 | 182                | 76          | 86                            | 2.3               |

Note: the calculations were made in relation to the total cell count.

For the time being, apart from *A. cepa*, the root meristem of many other higher plants such as barley (*Hordeum vulgare* L.), beans (*Vicia faba* L.) and corn (*Zea mays* L.) are used to study both toxic and genotoxic factors of different nature (Grant, 1994). However, due to the high proportion of mitosis cells, its high sensitivity and easiness (Leme & Marim-Morales, 2009), *A. cepa* has taken a clear lead as an experimental object, to the degree that Grant (1982) even suggested making the *Allium* assay the standard test for chromosome damage assessment. This test includes several parameters for proper estimation of cyto- and genotoxicity patterns such as root lengths (growth index); mitotic index; cell percentage in each mitosis phase; aneugenic and clastogenic effects.

Mitotic index reduction in the meristematic cells of *A. cepa* roots is related to the mitodepressive effect of tested substances (Akinboro & Bakare, 2007; Sharma & Vig, 2012). The ability of plant extract aqueous solution to inhibit cell proliferation, analogous to the one considered in this investigation, has been described earlier in studies of both potential and well-known medicinal and industrial plants (De Abreu et al., 2019; Mafe, 2007; Sudhakar et al., 2011). The discussed effect is caused either by DNA synthesis inhibition in the S-phase (El-Ghamery et al., 2000) or by cell-cycle blocking in the G2-phase and a faster passing of the prophase stage when the spindle apparatus is disrupted and pathological mitosis occurs (Karpova et al., 2020). The aberrations detain a cell in the metaphase, anaphase and telophase phases and lead to the increase of the mentioned indices. A similar indices shift has been observed in an earlier study of higher hogweed extract concentrations (Pesnya et al., 2017).

The mitosis changes and chromosome anomalies accounted for during the *Allium* assay are divided into two groups, known in the literature

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as aneugenic (lagging and sticking chromosomes, and nuclear buds) and clastogenic (ring chromosomes and anaphase bridges) (Sharma et al., 1990; Boncii, 2018). These changes are related to DNA rupture, chromo-
some decay, mitotic spindle disturbance and cytokinesis inhibition. Chro-
mosome lagging occurs due to the division spindle disturbance caused by 
inhibition of cytoskeletal proteins and tubulin polymerization (Timoshiev-
sky & Nazarenko, 2006; Boncii et al., 2018). Chromosome sticking, on 
the other hand, is a reaction to compound toxicity and often results in cell 
death. There are several degrees of chromosome sticking in anaphase and 
telophase (light, moderate and severe). In its classical understanding, the 
term refers to the severe degree when the chromosomes form an amorphic 
cluster (cluster) due to the functional defect of the specific non-histone pro-
teins organizing chromosomes in mitosis for chromatin segregation and 
division (Gaulden, 1987; Ribeiro, 2018). Nuclear buds, on the other hand, 
occur when chemical compounds affect a cell’s mitotic cycle and is rela-
ted to the processing following chromosome lagging when the last are 
engaged by the nuclear membrane earlier than by the pole chromosomes 
(Serrano-Garcia & Monteiro-Montoya, 2001). Some authors insist the 
buds occur due to polyphoidisation and amplification of the genetic ma-
terial that is removed from the nucleus but remains bound to the nuclear 
membrane (Fernandes et al., 2007; Fenech et al., 2011). Chromosome and 
chromatin sticking lead to clastogenic effects, in particular anaphase and 
telophase bridges. Such bridges can be the product of unbalanced chro-
mosome segment translocation or inversion (Kuras, 2004; Bonciu et al., 2018).

In our study, giant and enucleate (ghost) cells with damaged mem-
branes were observed in the root cap and root division zones. The giant 
cells form when the cells have entered mitosis but not yet finished the 
cytotplastic division (Kenne et al., 1986). The ghost cells, on the other hand, 
are dead cells in which the nucleus and cytoplasmic structures cannot be 
stained (Ribeiro, 2018). The membranes are damaged due to the effect of 
the membrane enzymes produced by lipid peroxidation or due to reducing 
cellulose content (Sultan & Celik, 2007, 2010).

Conclusion

Our study has demonstrated that all the concentrations of the H. sos-
novskyi extract aqueous solution prepared from fresh leaves had mito-

dressive effect on the meristic make of the A. cepa roots, which mani-

fested itself in statistically significant and concentration-dependent reducti-
on of root length and MI. In the 0.5 mL/L solution, the phase indices were 
moved in such a way that the proportion of prophase was reduced and 
that of metaphases, anaphases and telophases increased. A positive corre-
lation has been discovered between the extract concentration and the number of aberrant cells in mitosis. The aqueous solution’s effect was 
mainly aneugenic and manifested itself in lagging and sticking chromo-
somes, and nuclear buds. As for clastogenic aberrations, they included 
aneuphase bridges and ring chromosomes. Other changes included ghost 
and giant cells, and cells with disrupted membranes.

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References

Akinboro, A., & Bakare, A. A. (2007). Cytotoxic and genotoxic effects of aqueous 
extracts of five medicinal plants on Allium cepa L. Journal Ethnopharmacology, 
112(3), 470–475.

Alouo, N. A., Naidoo, Y., & Singh, M. (2019). Cytogenotoxic and biological evalua-
tion of the aqueous extracts of Grevia lasiocarpa: An Allium cepa assay. South 
African Journal of Botany, 123, 371–380.

Bahadori, M. B., Dinparast, L., & Zengin, G. (2016). The genus Heracleum: A com-
prehensive review on its phytochemistry, pharmacology, and ethnomedical 
values as a useful herb. Comprehensive Reviews in Food Science and Food 
Safety, 15(6), 1018–1039.

Bogacka-Kocka, A., Smoliarz, H., & Kocki, J. (2008). Apoptotic activities of ethanol 
extracts from some Acanthaceae on human leukemia cell lines. Fitoterapia, 79, 
487–497.

Boncii, E., Firbas, P., Fontanetti, C. S., Wuschen, J., Karaismailoglu, M. C., Liu, D., 
Meniaci, F., Pesary, D. S., Popescu, A., Romanovsky, A. V., Schiff, S., 
Slanuvczyk, J., De Souza, C. P., Sriravasta, A., Sultan, A. O., & Papini, A. (2018). An 
evaluation for the standardization of the Allium cepa test as cytotoxic-
ty and genotoxicity assay. Cytology, 71(3), 191–209.

Christopher, H. B., & Kapoor, M. B. (1988). The cytogenetic effects of sodium Salici-
ylate on the root meristem cells of Allium sativum L. Cytologia, 54, 203–209.

Cock, M. J. W., Eikelstein, L., Evans, H. C., & Fröberg, L. (2007). Ecology and mana-
gement of giant hogweed (Heracleum mantegazzianum). CABI, Institute of 
Botany, Academy of Sciences of the Czech Republic, repozit.

De Abreu, J. C., De Santanna, R. A., Ribeiro, G. R. H., Dantas, M. M., Guiairo, L. F., 
Valente, L. L., Luiz Silva, W. S., Silva, D. R., Barbieri, R. S., & Da Costa, N. M. (2019). Effects 
cytotoxic and genotoxic of aqueous extract of fennel (Foeniculum vulgare var. vulgare MILL). International Journal of Advanced En-
ingineering Research and Science, 6(3), 230–236.

El-Ghamery, A. A., El-Nahas, A. I., & Mansour, M. M. (2000). The action of atrac-
temical that is removed from the nucleus but remains bound to the nuclear 
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Alouo, N. A., Naidoo, Y., & Singh, M. (2019). Cytogenotoxic and biological evalua-
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