CYTOGENETIC ANALYSIS ON THE EFFECT OF *ROSA GALLICA* L. WASTEWATER GENERATED DURING WATER-STEAM DISTILLATION IN THREE TYPES OF TEST- SYSTEMS

Svetla Gateva[1], Gabriele Jovtchev[1], Tsveta Angelova[1], Tsvetelina Gerasimova[1], Margarita Topashka-Ancheva[1], Ana Dobreva[2], Milka Mileva[3]

[1] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 2 Gagarin Str., Sofia1113, Bulgaria
[2] Institute for Roses and Aromatic Plants, 49 Osvobojdenie Blvd, Kazanlak 6100, Bulgaria
[3]The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 26 Acad. G. Bonchev Str., Sofia 1113, Bulgaria

ICE 2023, 9-10 June

Background:

***Rosa gallica* L.**

***Rosa gallica* L.**, known as **Gallic rose**, or **French rose** (Rosaceae) is characteristic of Central and Southern Europe including Bulgaria.

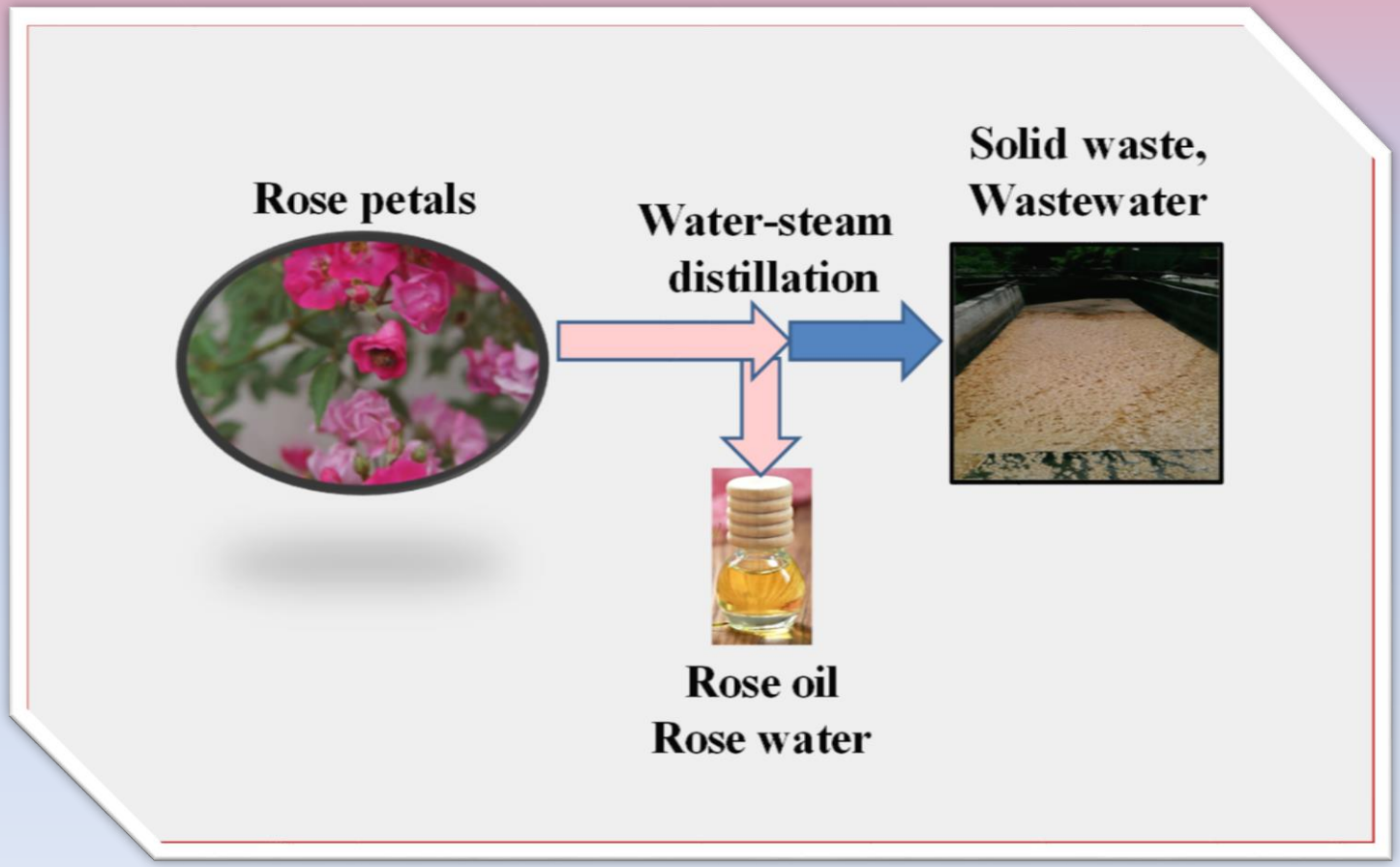
It is one the oldest rose species and is a parent of many cultivars such as *Rosa damascena* Mill.

Essential oil and hydrosol are the main rose products, which are used in perfumery, pharmacy, cosmetics, medicine, and aromatherapy.

Biological activities of *R. gallica* L. essential oil and hydrosol :

- anti-inflammatory
- antibacrterial
- antioxidant
- suppress cancer cell proliferation
- hydratation and moisturization
- anti-aging effect of skin
- cleansing properties
- antidepresive effect
- relieve arthritic pain

Wastewater is the main product discarded during the water-steam distillation of rose oil. The amount released into the environment is in the range of tones and can cause soil pollution. Hence it is essential to test the safety of rose wastewater.



Aim:

The main goal of the study was to investigate the cytotoxicity and genotoxicity of ***Rosa gallica* L. wastewater** generated during the water-steam distillation of rose oil in three different types of test-systems (higher plant - *H. vulgare*, ICR mice *in vivo* and human lymphocytes *in vitro*).

For this purpose, two cytogenetic tests were applied:
i) for induction of chromosome aberrations;
ii) for micronuclei.

Material and methods:

Wastewater extraction

The wastewater was obtained by a semi-industrial water–steam distillation cycle of *Rosa gallica* L. oil at the Institute for Roses and Essential Oil Plants in Kazanlak, Bulgaria (growing season of 2022). The process parameters were as follows: raw material 8–10 kg; hydromodule 1:4; flow rate 16–20 mL/min; duration 150 min.

Chromatographic analysis

The chromatographic profile of the rose wastewater was obtained by UHPLC-HRMS/MS analysis using a Thermo Scientific Dionex Ultimate 3000 RSLC.

Material and methods:

The content of tannins and flavonoids was established according to the European Pharmacopoeia method (COE, 2013) and is expressed as mg/mL of pyrogallol equivalents for tannins, and as mg/mL of hyperoside equivalents for flavonoids.

The total phenolic content was determined by Folin–Ciocalteu method with some modifications (Zheng and Wang, 2001). Their quality was evaluated as gallic acid equivalents per 1 mL of WW (µg GAE/ mL) based on a standard curve of gallic acid.

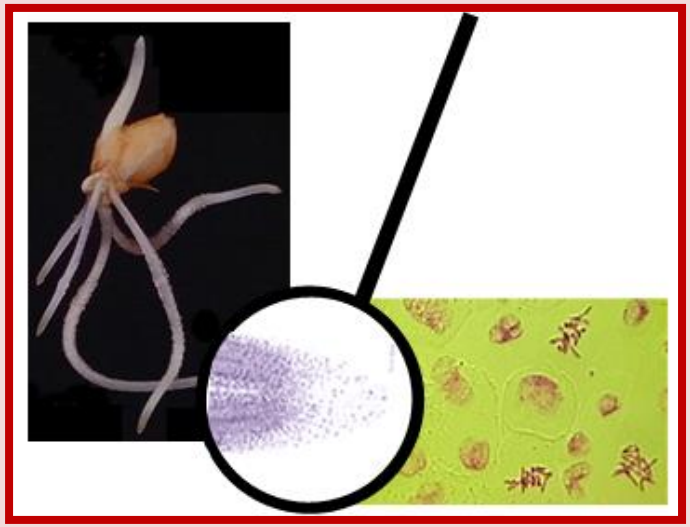
Tannins (mg/mL)	Total flavonoids (mg/mL)	Total polyphenols (mg/mL)
1.51 ± 0.09	**0.37 ± 0.02**	**7.7 ± 0.03**

Georgieva et al., (2021)

Material and methods:

Test-systems

Higher plant – ***Hordeum vulgare*** **(barley)** reconstructed karyotype MK14/2034 (which has seven easily distinguishable chromosome pairs) was used. Presoaked barley seeds (1 h in tap water) germinated in Petri dishes on moist filter paper at 24°C. Root tip meristems were treated with *R. gallica* L. wastewater at concentrations of 6, 14, and 20 % for 4 hrs.

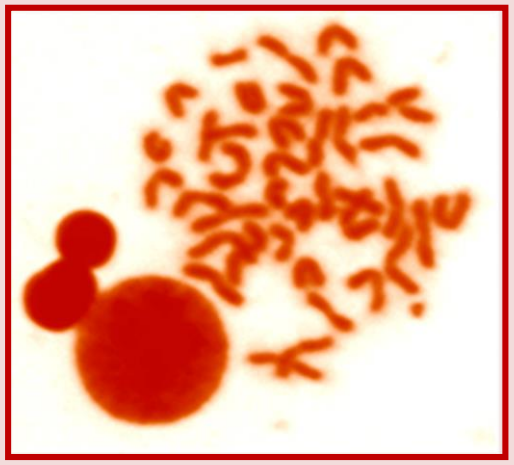


Material and methods:

Animal test-system *in vivo*

Male and female **ICR strain albino** mice were used in the experiments. The animals were randomly allocated to four experimental groups (eight male/eight female) and kept in standard cages.

The rose wastewater was given as a single treatment by *i. p.*injection. One group of animals was injected with 11 %, the second with 20%, the third one with genotoxin MNNG, and the fourth one was a negative control. The cytogenetic protocol of Preston et al. (1987) was applied.

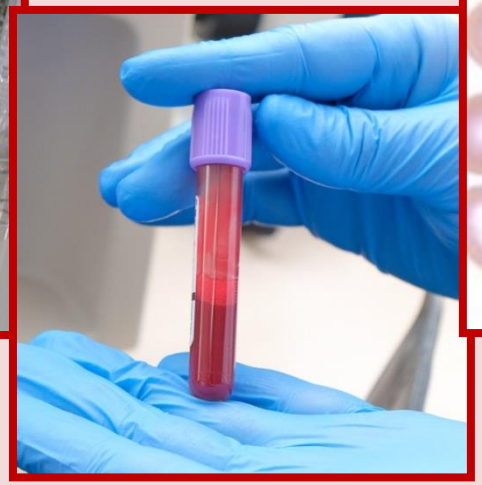


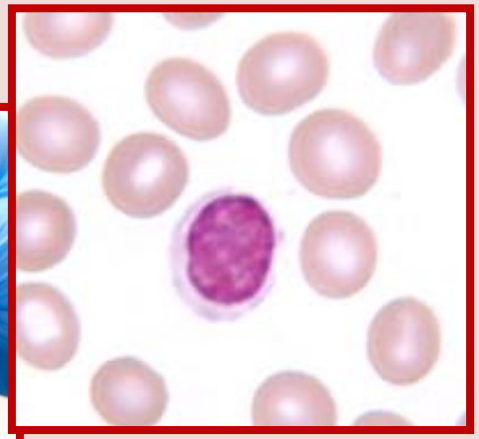
Material and methods:

Human lymphocytes *in vitro*

Lymphocyte cultures were prepared from the venous blood of healthy donors (aged 33 - 40 years). Each culture contained 3.5 ml RPMI medium with 12% fetal calf serum, 40 mg/mL gentamycin, and 0.1% phytohemagglutinin (PHA).

The lymphocytes were treated with *R. gallica* L. wastewater in concentrations of 6%, 11%, 14%, and 20% for 4 hrs. The cytogenetic protocol of Evans (1983) was applied.





Material and methods:

For genotoxicity, the percentage of metaphases with chromosome aberrations **(MwA%±SD)** and micronuclei **(MN%±SD**), respectively were calculated.

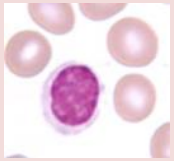
For cytotoxicity, mitotic index **(MI)** was calculated **(MI=A/1000).**
Standard mutagen N-methyl -N'-nitro -N-nitrosoguanidine (MNNG) was used as a positive control. Untreated samples were negative control.
Each experiment was repeated three times.

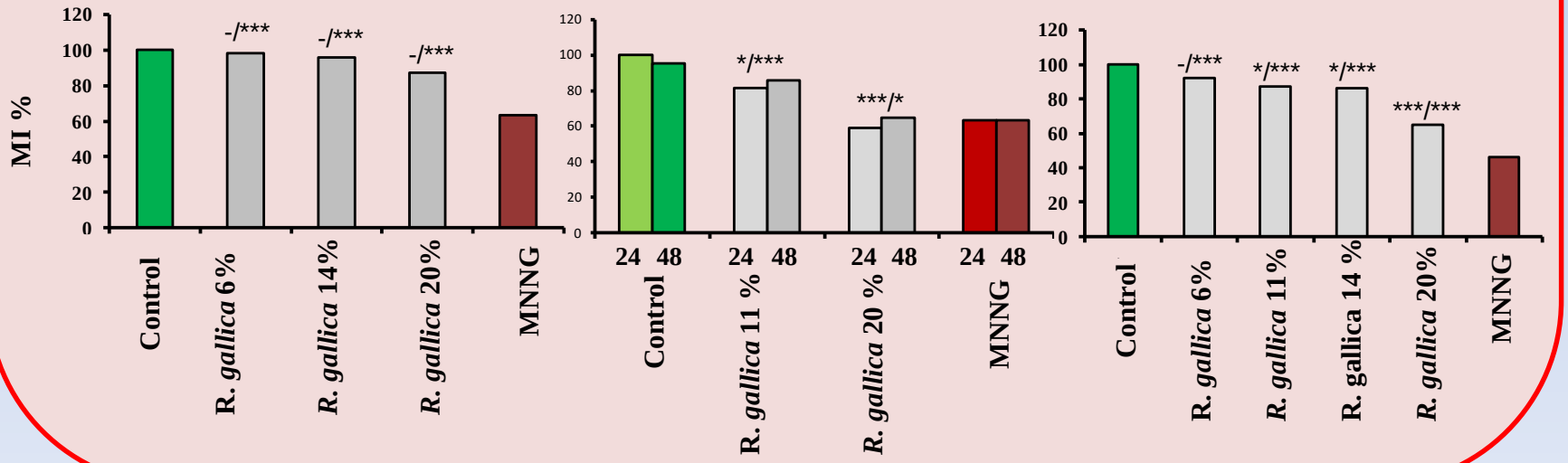
The results were assessed statistically by one-way ANOVA with a two-tailed Fisher's exact test.

Results:

Cytotoxicity

The rose wastewater has no **cytotoxic effect (MI)** in *H. vulgare*, whereas only the highest concentration (20%) induced a decrease in the mitotic activity both in ICR mice and lymphocytes.





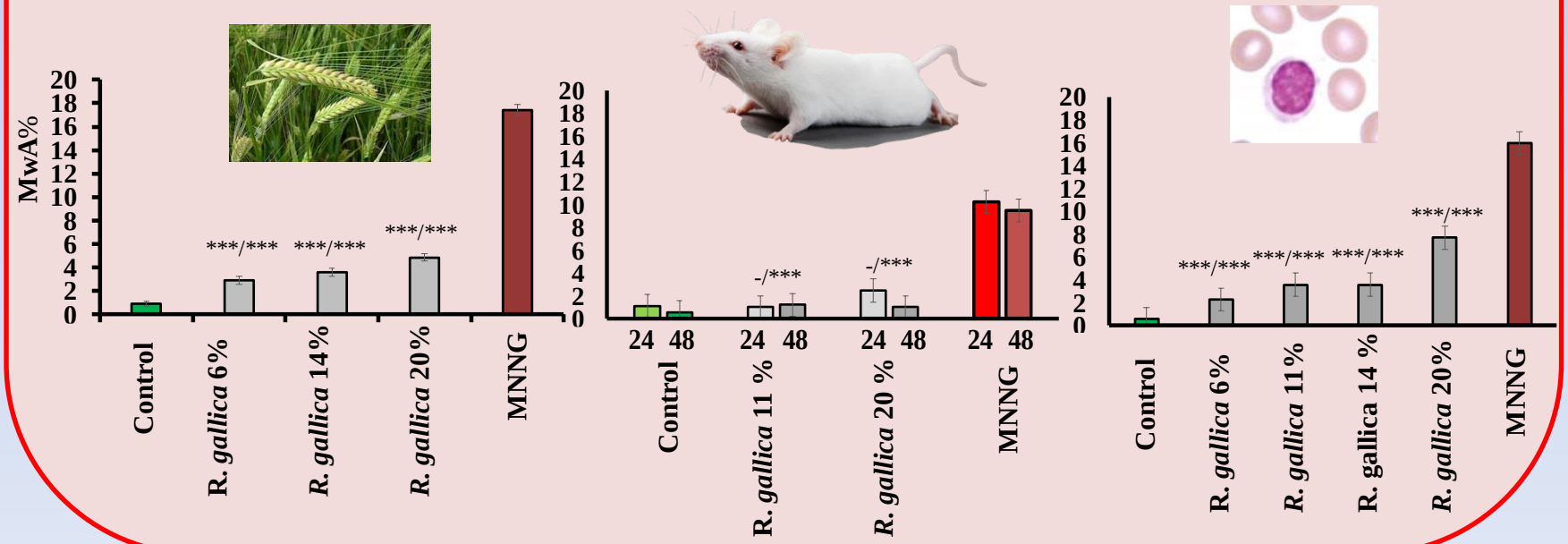
Results:

Genotoxicity

Rose wastewater induced a low, but statistically significant genotoxicity for the concentrations applied in plant and lymphocyte test-systems, whereas no damaging effect was observed in mice cells calculated by induction of **chromosome aberrations (MwA).**

No concentration dependence was obtained for the value of aberrations both in barley and ICR mice. The same is observed in lymphocytes for concentrations lower than 20%.

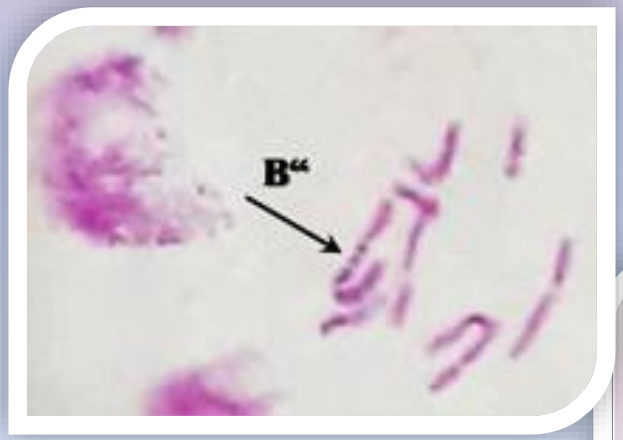
Lymphocyte cells were more sensitive than the other two test-systems.



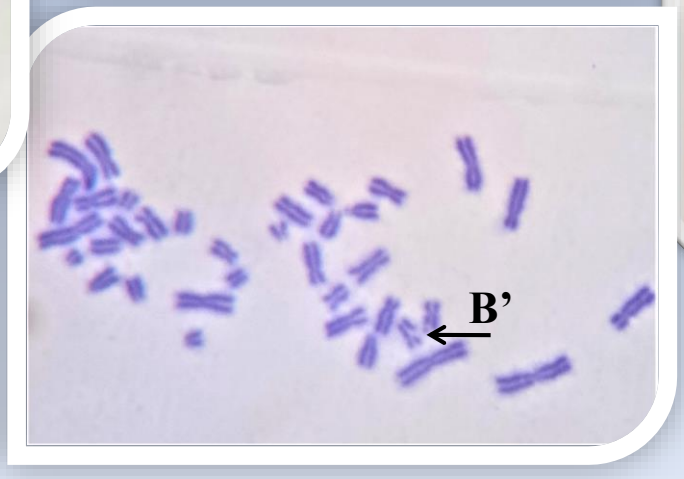
Results:

Genotoxicity

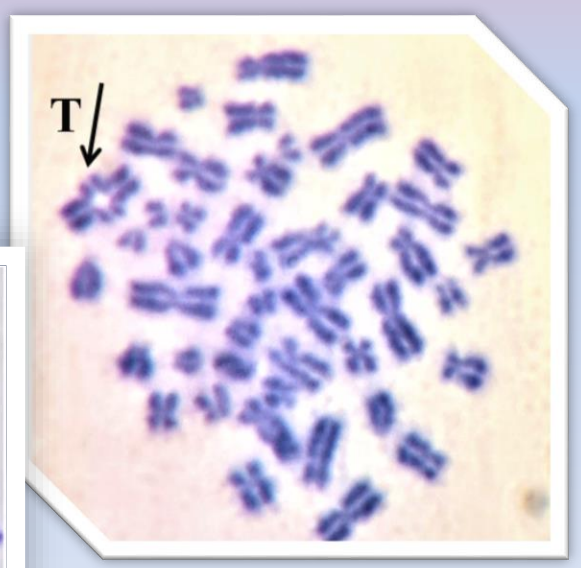
Chromosome aberrations induced in barley and human lymphocytes by *R. gallica* L. wastewater were mainly isochromatid breaks **(B")** and chromatid breaks **(B').** In samples treated with the highest concentration 20% translocations **(T)** were observed. In mice bone marrow cells, predominantly centromere–centromeric fusions followed by breaks and fragments were detected.



Isochromatid break (B")



Chromatid break (B")



Translocation (T)

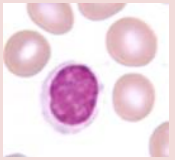
Results:

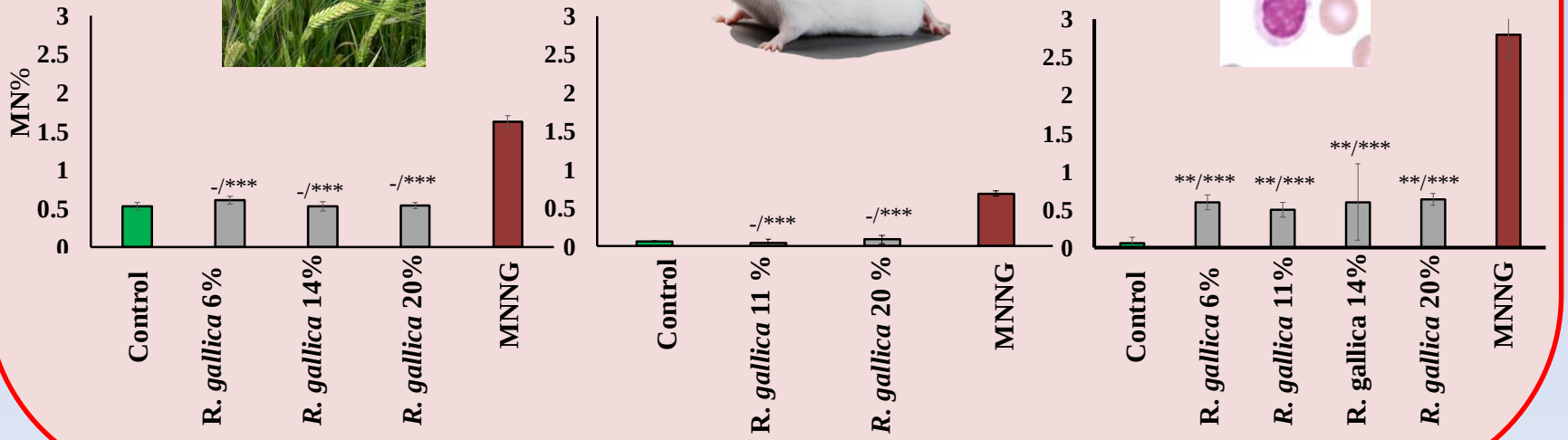
Genotoxicity

No enhanced values of **micronuclei (MN)** were observed both in barley and in ICR mice after treatment with *R. gallica* L. wastewater.

Lymphocyte cultures demonstrated higher sensitivity, as all wastewater concentrations (6%-20%) induced similar micronuclei values that are higher ($p < 0.001$) than those of the negative control.

Genotoxic effect of the wastewater assessed by MN in all cell types is much lower than that of MNNG.

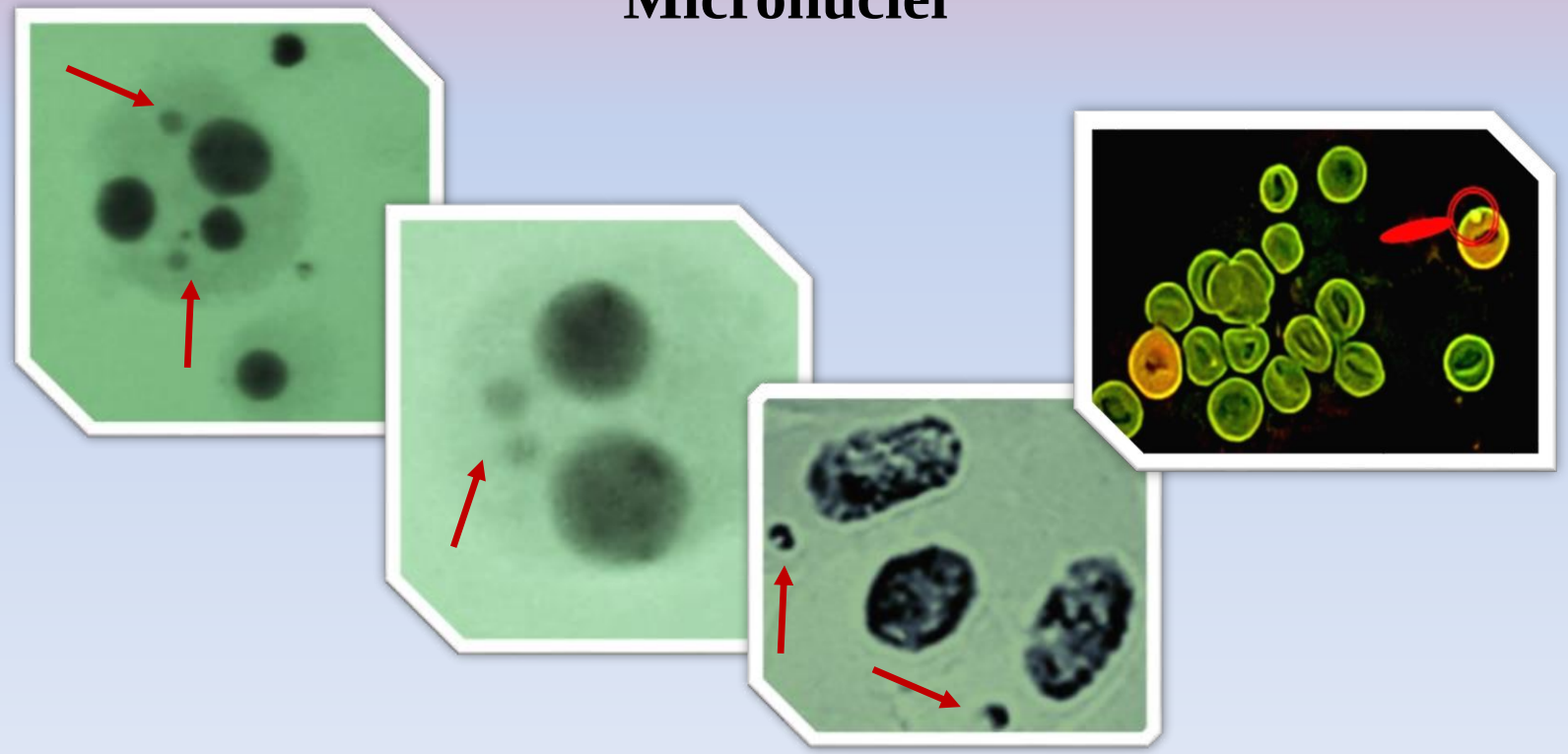




Results:

The low values of micronuclei in *H. vulgare* and ICR mice are an indicator that rose wastewater did not induce an aneugenic effect in these test-systems and induced a low such in human lymphocytes *in vitro*.

Micronuclei



Conclusions:

- The waste product induced no or very low cytotoxic/genotoxic effect clearly depending on the test-system sensitivity and the concentration applied.
- The cytotoxic/genotoxic effect is much lower than that of the direct mutagen MNNG.
- Human lymphocytes *in vitro* show the highest sensitivity to the wastewater compared with the other two test-systems.
- The present results demonstrate that the wastewater generated during the water-steam distillation of *R. gallica* L. essential oil does not possess a harmful effect. These data as well as the presence of valuable biological activity compounds, show its good potential to be used in human life.

This work is supported by the Project of Bulgarian National Science Fund № КП-06 -Н36/17 (granted to Assoc. prof. M. Mileva PhD).


Thank you for your attention