Genotoxic screening of the effect of *Rosa gallica* L. essential oil evaluated by induction of chromosome aberrations and micronuclei in plant and human lymphocyte test systems

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Introduction

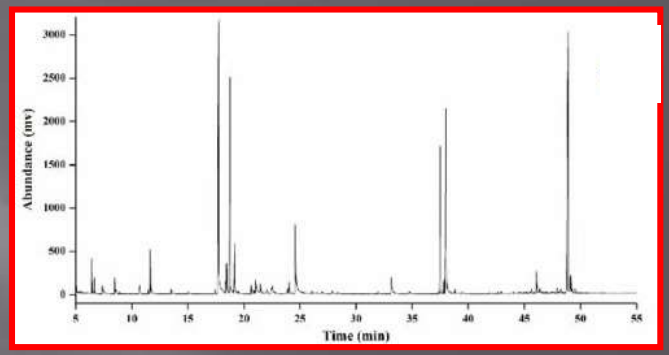
Rosa gallica L., also known as the French rose, is widely spread in various European countries including Bulgaria.

The traditional breeding region in our country is the Rose Valley, near the town of Kazanlak.

The main rose product - essential oil finds application in folk medicine, perfumery, cosmetics and culinary.

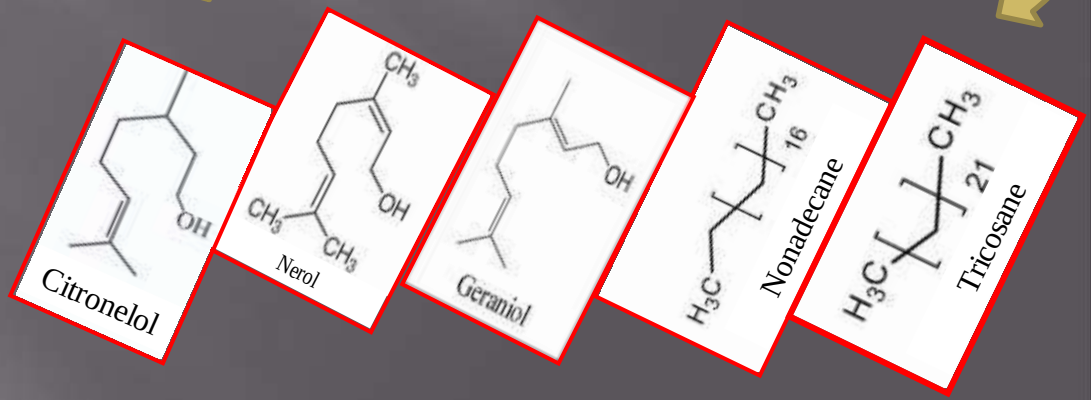
R. gallica essential oil is produced by the classical method of water-steam distillation.

GC-FID/MS analysis of the essential oil



The oil contains 220 phytochemicals from different chemical classes in different quantitative ratios.

Only twenty of them are in great abundance (Dobreva et al., 2023).



Structures of some of the main chemical compounds

Aim

Limited studies exist about the cytotoxic/genotoxic activities of *R. gallica L.* oil. Its widespread use in human practice and the presence of numerous compounds warrant the need to assess its safety and non-toxic concentrations.

The present study aimed **to assess the cytotoxic and genotoxic/clastogenic activity of the *R. gallica* L. oil** using tests for induction of chromosome aberrations and for micronuclei in higher plant *H. vulgare* (barley) and human lymphocytes *in vitro*.

Material and methods

Fresh rose blossoms were picked early in the morning (6-8 a.m.). Rose oil was obtained by water vapour distillation in a Clevenger-type apparatus at the Institute for Rose and Aromatic Plants, Kazanlak.

Test-systems

H. vulgare - karyotype MK14/2034. Barley root meristems were prepared according to the method of Jovtchev et al., (2002)

Human lymphocyte cultures were prepared according to the method of Evans, (1984)

Material and methods

Endpoints

For cytotoxicity

i) mitotic index **(MI)** (Jovtchev et al., 2010),
MI=A/1000, A-number of mitosis

ii) nuclear division index **(NDI)** in lymphocytes (Fenech, 2007)**,**
NDI=N1+N2+N3+N4/N, where N1–N4 number of cells with 1–4 nuclei,
N - total number of scored cells.

For genotoxicity

i) chromosome aberrations **(CA)** (Evans, 1983; Jovtchev et al., 2010)
ii) micronuclei **(MN)** (Fenech, 2007).

Statistics

One-way ANOVA with a two-tailed Fisher's exact test.

Material and methods

Treatment

***R. gallica* oil**

100µg/ml
200µg/ml
500µg/ml

50µg/ml
100µg/ml
500µg/ml

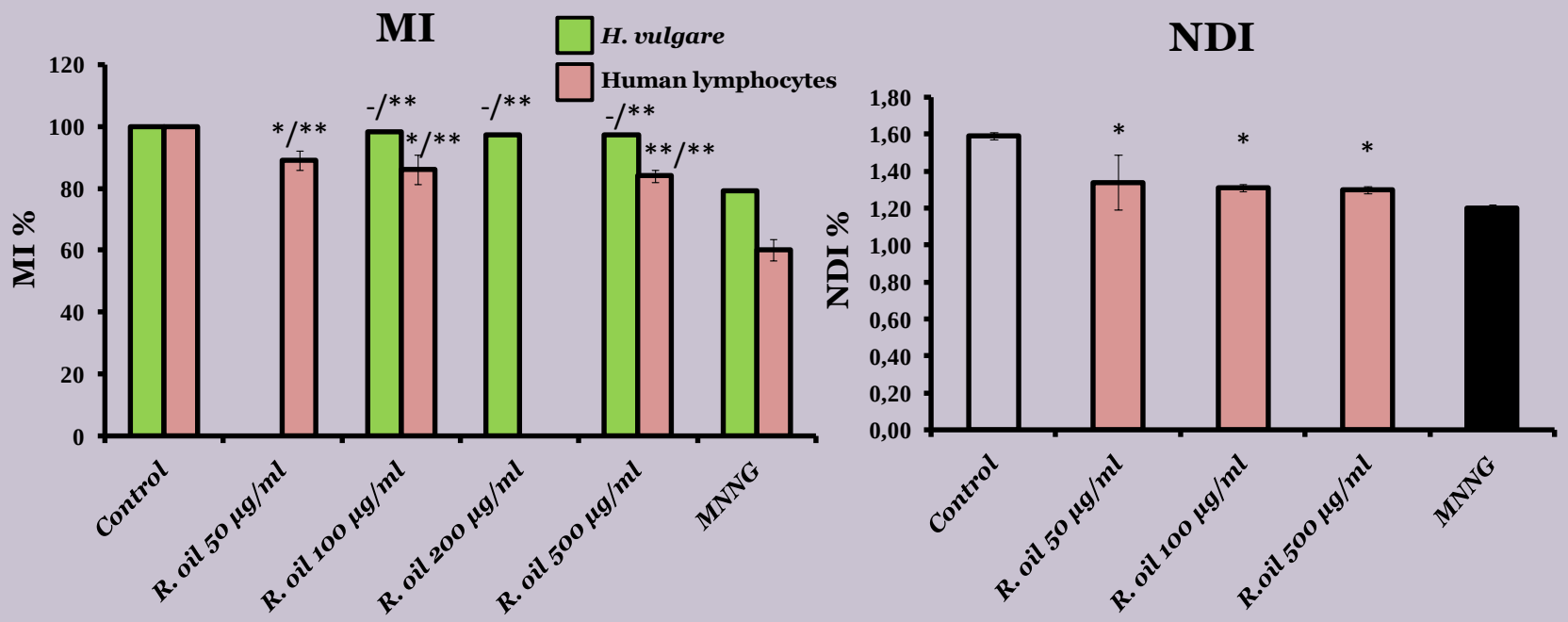
barley

lymphocytes

Fixation

Results

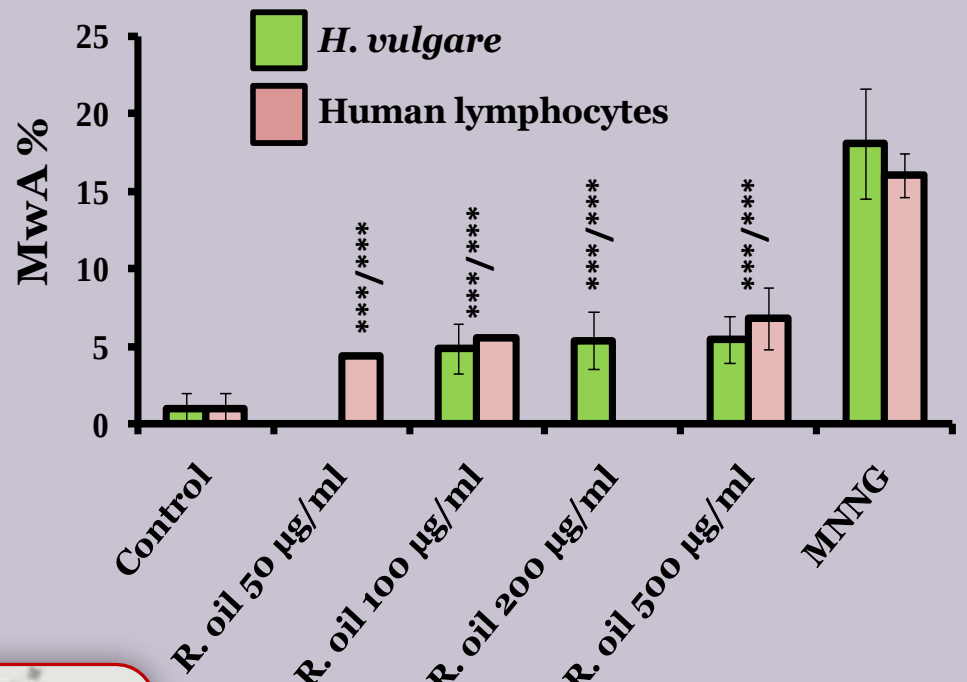
Cytotoxic activity

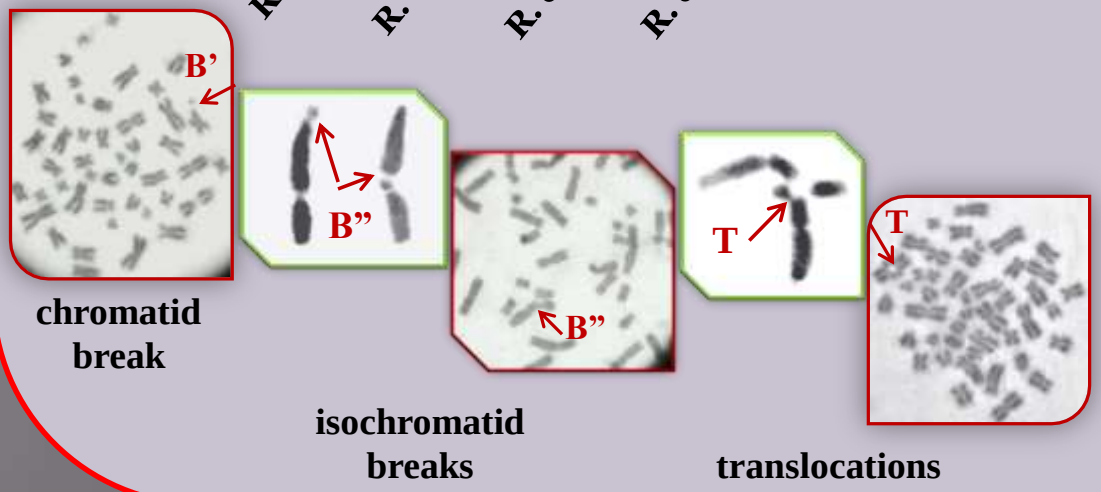


- The essential oil was not cytotoxic for barley cells, while the mitotic index measurements (MI) indicated low cytotoxicity in lymphocytes ($p<0.05$, $p<0.01$) compared to the untreated control.
- The values of NDI calculated in lymphocyte cells showed no high cytotoxic activity.
- The cytotoxic effect of rose essential oil (MI and NDI) was statistically lower than that of the direct mutagen MNNG (50µg/ml) used as a positive control in barley and human lymphocytes.

Results

Genotoxic activity - CA





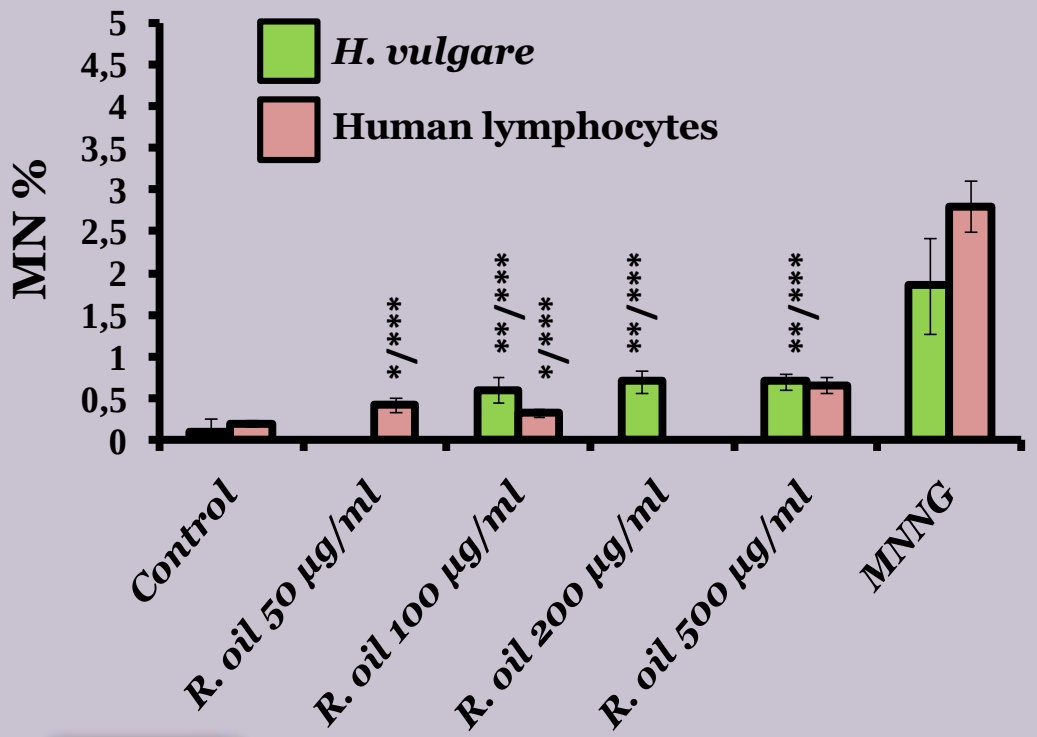
✓ The rose product had low but statistically significant genotoxic activity ($p<0.001$) as increased chromosome aberrations **(CA)** in both test systems used.

✓ No concentration dependence was obtained for the frequency of aberrations in barley, whereas in the lymphocyte cells, such an effect was registered.

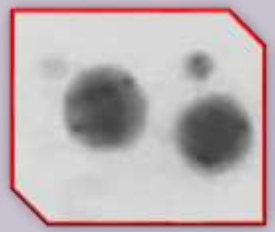
✓ Rose oil showed much lower genotoxic activity ($p<0.001$) than MNNG (50µg/ml) in barley and in human lymphocytes *in vitro*.

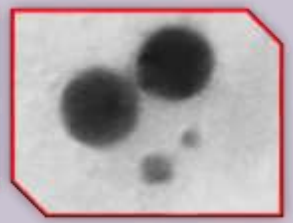
Results

Genotoxic activity - **MN**



Cell with spindle defects





Cells with MN

- ✓ Treatment with oil concentrations (50-500 µg/ml) induced **low** but statistically significant values ($p<0.05$, $p<0.01$) of MN compared with the untreated samples.
- ✓ **No concentration dependence** was registered for the frequency of micronuclei in barley cells and human lymphocytes.
- ✓ The genotoxic effect of the rose oil assessed by micronuclei induction **was lower** ($p<0.001$) than that of MNNG, as it was also obtained with a CA endpoint.

Conclusions:

R. gallica essential oil demonstrated weak cytotoxic and genotoxic activities in the tested concentrations in two different experimental test systems.

The obtained effects were much weaker than that of the genotoxin MNNG.

The obtained data reveal the safety concentrations and conditions for this rose product application and would be useful for further use in human life.

Thank you for your attention!

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