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IN VIVO AND *IN VITRO* CHARACTERISATION OF THE CYTOTOXIC AND GENOTOXIC EFFECTS OF *ROSA DAMASCENA* MILL. EXTRACT DERIVED BY SUBCRITICAL WATER EXTRACTION

Svetla Gateva[1], Gabriele Jovtchev[1], Tsvetelina Gerasimova[1], Milka Mileva[2], Ana Dobreva[3]

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

ABSTRACT

The application of new so-called "green" extraction technologies is essential for the safety of plant essential oils and extracts, and for their beneficial effects on human health. Subcritical water extraction is a new eco-friendly technology for the extraction of active substances from the plants contributes to more effectively extraction of biologically active compounds in the extracts. *R. damascena* Mill. hydrosol, additionally with the rose essential oil, is highly valued and used in perfumery and numerous areas of life. There is a lack of analysis about the biological effect of the rose extracts derived by this method.

The goal of the study was to assess the cytotoxic/genotoxic potential of different concentrations of *R. damascena* extract derived by this "green" technology in three types of test systems (barley, laboratory mice and human lymphocytes) by applying cytogenetic test for analysis.

Phytochemical analysis of the extract showed the presence of a large amount of polyphenols - phenolic acids, flavonoids and glycosides of flavonoids. The rose extract does not show statistically significant cytotoxic/genotoxic effect without any dose dependence, both in barley at concentrations 6%-20%, and in laboratory mice at concentrations 11%-20%. Lack of cytotoxicity and a very low genotoxic effect after application of the rose extract (6%-20%) were observed in human lymphocyte cultures *in vitro*. The results show that the obtained effect depends on the phytochemical components of the extract, whose composition directly depends on the extraction technology used. The extract could find good application in various areas of human life, including medicine.

INTRODUCTION

Subcritical water extraction technology developed recently is promising for more effective extraction of valuable biologically active compounds from the plants. *R. damascena* Mill. hydrosol and essential oil obtained by classical steam distillation play an essential role in perfumery and numerous areas of life. There is a lack of analysis about the biological activities, including the cyto/genotoxic effects of the rose extracts derived by the new “green” technology.

Aim: to assess the cytotoxic/genotoxic potential of different concentrations of *R. damascena* extract derived by "green" technology in three different types of test systems, applying a test for genotoxicity-induction of chromosome aberrations.

MATERIAL AND METHODS

***R. damascena* Mill. *extract*:** Fresh rose flowers from the experimental field of the Institute for Rose and Aromatic Plants, Kazanlak, Bulgaria (harvest season of 2024) were used. The extract was generated during the subcritical water extraction using water at high temperature and high pressure. The chromatographic analysis was performed on an HPLC system (Thermo Scientific Spectra SYSTEM) equipped with UV2000 and FL3000 detectors.

Test-systems: ***Hordeum vulgare*** root tip meristem cells, reconstructed karyotype MK14/2034 prepared according to Jovtchev et al., (2002). Used concentrations of rose extract - 6, 14, 20 % for 4 hrs.

ICR strain mice were utilized according to the method of Preston et al. (1987). A group of animals were *i. p.* injected with 11 % of rose extract, second with 20% for 24 hrs and 48 hrs.

Human lymphocyte cultures were prepared from venous blood of healthy donors, (35-36 years old) according to Evans, (1984). The concentrations used were 6, 14, 20 % for 4 hrs.

Endpoints for: cytotoxicity - mitotic index **MI=A/1000**

genotoxicity- chromosome aberrations **MwA%±SD (Fig. 1)**

Statistics: One-way ANOVA with a two-tailed Fisher’s exact test.

RESULTS

- Phytochemical analysis of the extract showed the presence of 0.02 - 0.06 % essential oil (containing phenylethyl alcohol, a large amount of monoterpenoids, benzenoid compounds, aliphatic hydrocarbons), polyphenols - phenolic acids, flavonoids and glycosides of flavonoids (Fig. 2).
- The rose extract did not show a statistically significant cytotoxic/genotoxic effect without any dose dependence both in barley with concentrations of 6%-20% (Fig. 3, A, D), and in laboratory mice with concentrations of 11%-20%. (Fig.3, B, E) compared with the control.
- Lack of cytotoxicity (Fig.3, F) and a very low genotoxic effect (Fig.3, C) after application of the rose extract (6%-20%) were observed in human lymphocyte cultures *in vitro*.
- The effect of this rose extract in the applied concentrations is much lower than that of the positive control - mutagen MNNG ($p<0.001$).

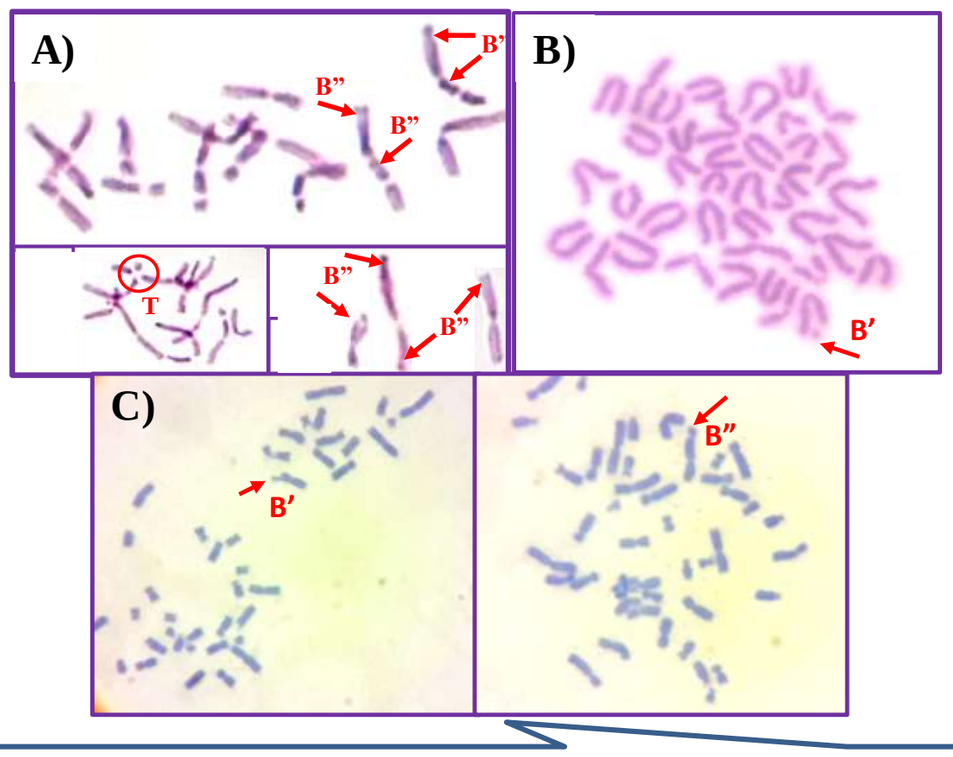

Fig. 1 Chromosome aberrations observed after treatment with *R. damascena* Mill. extract derived by subcritical water extraction in: A) *H. vulgare*, B) laboratoty mice, C) human lymphocytes. B” isochromatid break, B’ chromatid break

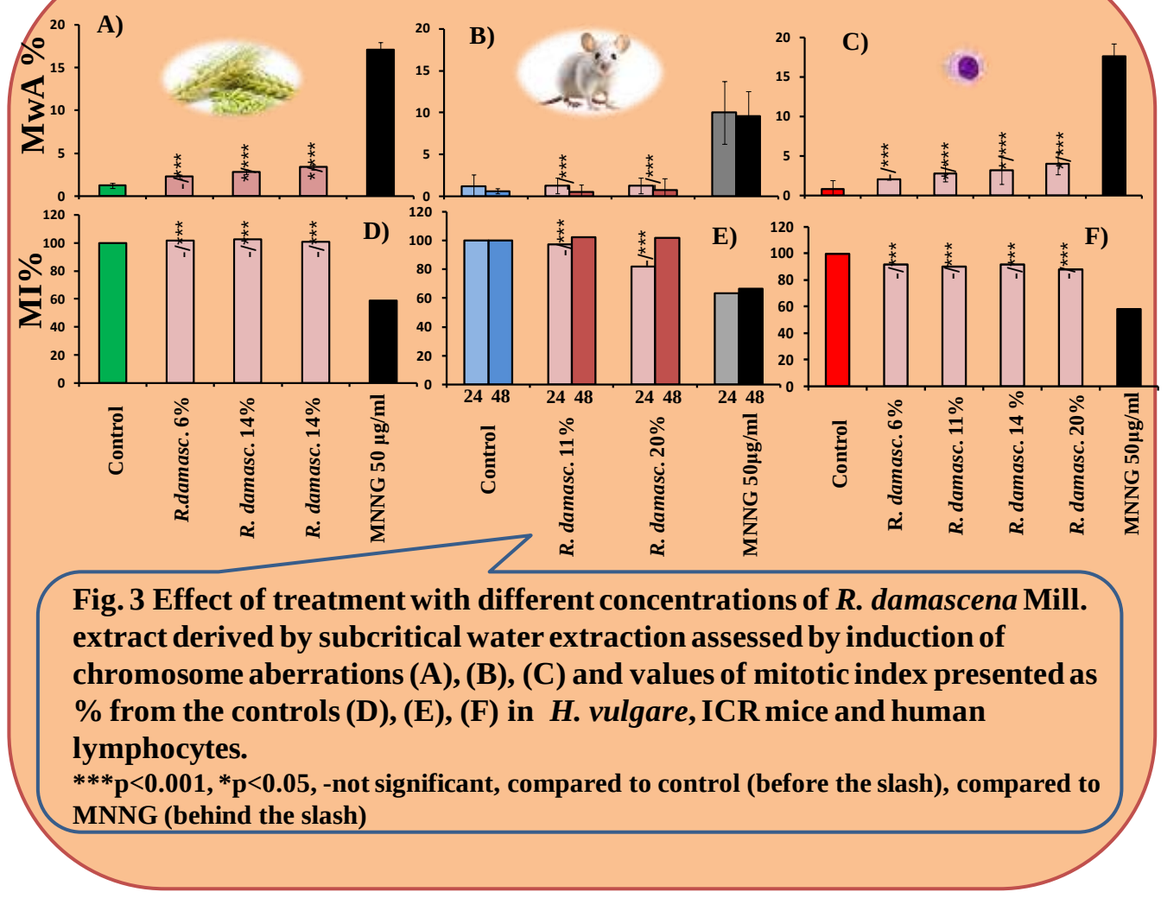

Fig. 3 Effect of treatment with different concentrations of *R. damascena* Mill. extract derived by subcritical water extraction assessed by induction of chromosome aberrations (A), (B), (C) and values of mitotic index presented as % from the controls (D), (E), (F) in *H. vulgare*, ICR mice and human lymphocytes.
*****p<0.001, *p<0.05, -not significant, compared to control (before the slash), compared to MNNG (behind the slash)**

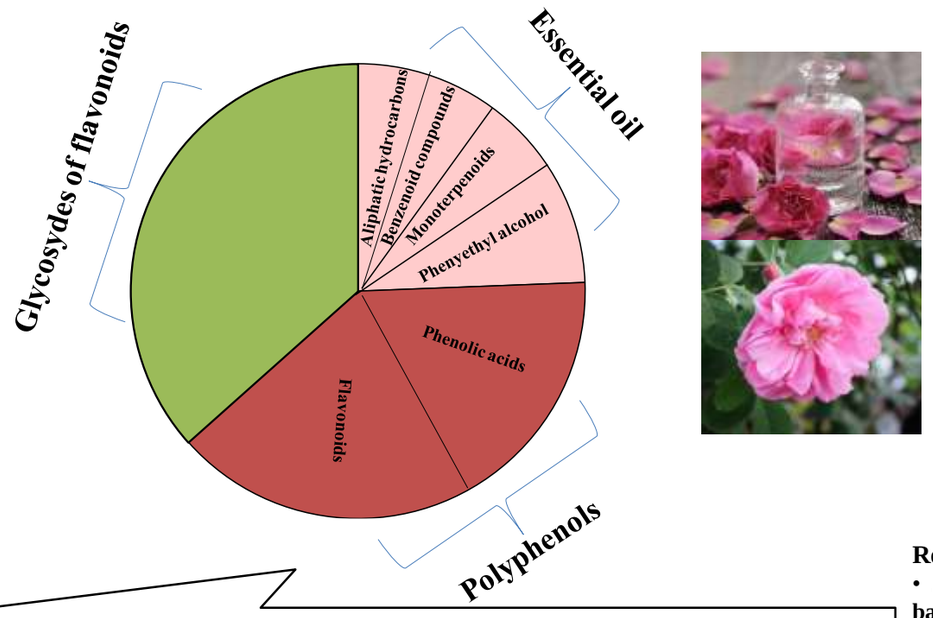

Fig. 2 Chemical composition of *R. damascena* Mill. extract derived by subcritical water extraction

CONCLUSION

The results show that the obtained effect of the rose extract depends on the phytochemical components of the extract, whose composition directly depends on the extraction technology used.

The extract derived by this modern “green” technology could find good application in various areas of human life, including medicine.

References:

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