Assessment of the cytoprotective and genoprotective activities of subcritical water extract of *Rosa alba* L. flowers in two different types of test-systems

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INTRODUCTION

Rosa alba L. is a primary oil-bearing rose species cultivated in Bulgaria, renowned for its essential oils and extracts used extensively in cosmetics and perfumery. The biological efficacy of these products is highly dependents on the extraction method, which determines their phytochemical profile. Recently, subcritical water extraction has emerged as an eco-friendly, cost-effective, and efficient alternative for isolating biologically active compounds.

The present study was focused on the investigation of the cytoprotective and genoprotective activities of subcritical water extract of *R. alba* L. flowers (SWE) against alkylating mutagen N-methyl- N-nitro- N-nitrosoguanidine (MNNG) using cytogenetic tests in plant and human lymphocyte test-systems.

METHODS AND MATERIALS

R. alba L. extract were prepared from fresh flowers grown in the experimental field of the IRAP, Kazanlak, Bulgaria.

The SWE was generated during the subcritical water extraction using water at high temperature and pressure (Fig.1). The phytochemical composition was performed on an GC-MS technique.

Test-systems: ***Hordeum vulgare*** root tip meristems, karyotype MK14/2034 (Jovtchev et al., (2002), ***human lymphocytes*** from venous blood of healthy donors (Evans, 1984).

Experimental designs: (i) a conditioning with non-toxic concentrations of SWE followed by a 4-hour recovery period before MNNG exposure, (ii) a co-treatment of SWE and MNNG without an inter-treatment period.

Endpoints: for cytotoxicity-mitotic index (MI), nuclear division index (NDI), for genotoxicity-chromosome aberrations (CA), micronuclei (MN) (Fenech, 2007).

RESULTS

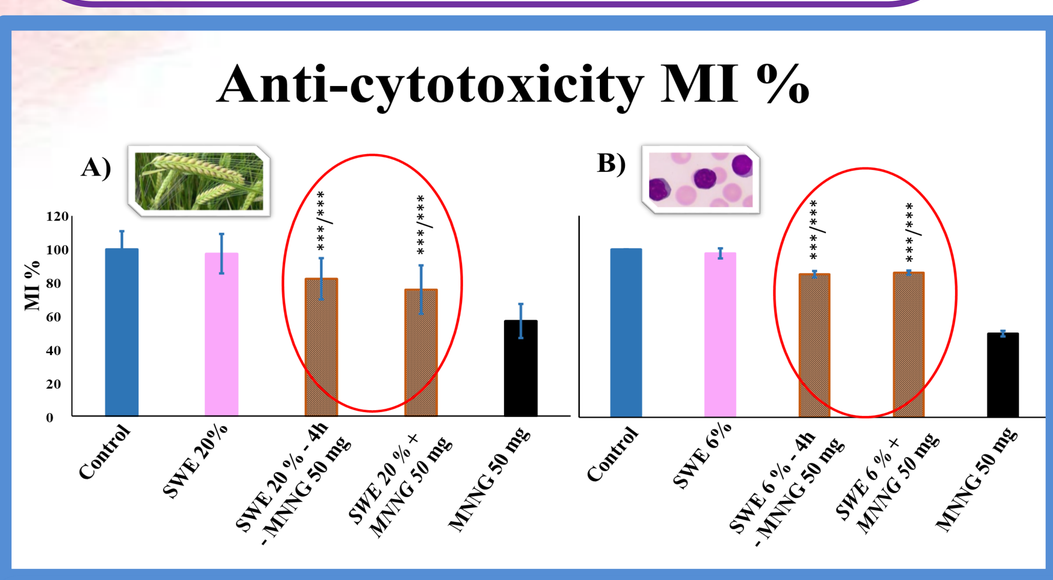

Figure 2. Anti-cytotoxic effect of *R. alba L.* SWE assessed by value of mitotic index (MI) after co- treatment with non-toxic concentrations of extracts and damaged concentration of MNNG with or without inter-treatment time in: A) *H. vulgare*, and B) human lymphocytes *in vitro*, ***p<0.001, before slash-compared to control, after slash –compared to MNNG; MI of treated variants is present as % of untreated control.

The major constituents of the *R. alba* L. flowers SWE, identified by GC-MS analysis, were as follows: monoterpene alcohols (geraniol, citronellol, nerol, linalool), phenylethyl alcohol, eugenol, methyl eugenol, and aliphatic hydrocarbons (data not shown).

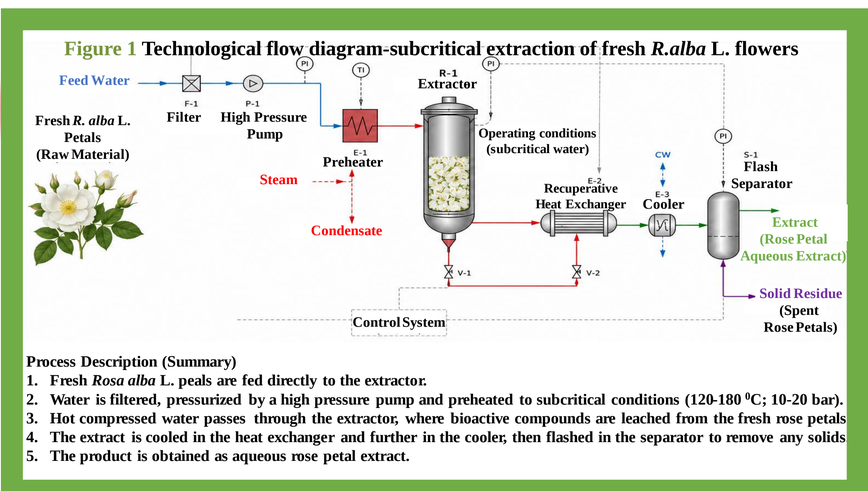

Figure 1 Technological flow diagram-subcritical extraction of fresh *R.alba* L. flowers

Process Description (Summary)

1. Fresh *Rosa alba* L. peals are fed directly to the extractor.
2. Water is filtered, pressurized by a high pressure pump and preheated to subcritical conditions (120-180 ^{0}C; 10-20 bar).
3. Hot compressed water passes through the extractor, where bioactive compounds are leached from the fresh rose petals
4. The extract is cooled in the heat exchanger and further in the cooler, then flashed in the separator to remove any solids
5. The product is obtained as aqueous rose petal extract.

The results point to a robust anti-cytotoxic effect of the SWE. The mitotic activity (MI), which was reduced ($p < 0.001$) by treatment with the mutagen alone, was restored under the respective schemes of combined treatment in both test systems (Fig. 2).

The similar restoration was observed for proliferative activity (NDI) in lymphocytes (data not shown).

The extract exposure demonstrated genoprotective potential, reducing MNNG-induced chromosomal aberrations (CA) by 2- to 3-fold across both test systems under combined treatment with *R. alba* SWE and MNNG (Fig. 3 A, B).

A significant decrease in MN frequency ($p < 0.001$) was also observed, while the protective effect in lymphocyte cells was slightly more pronounced than in the plant model (Fig. 3 C, D).

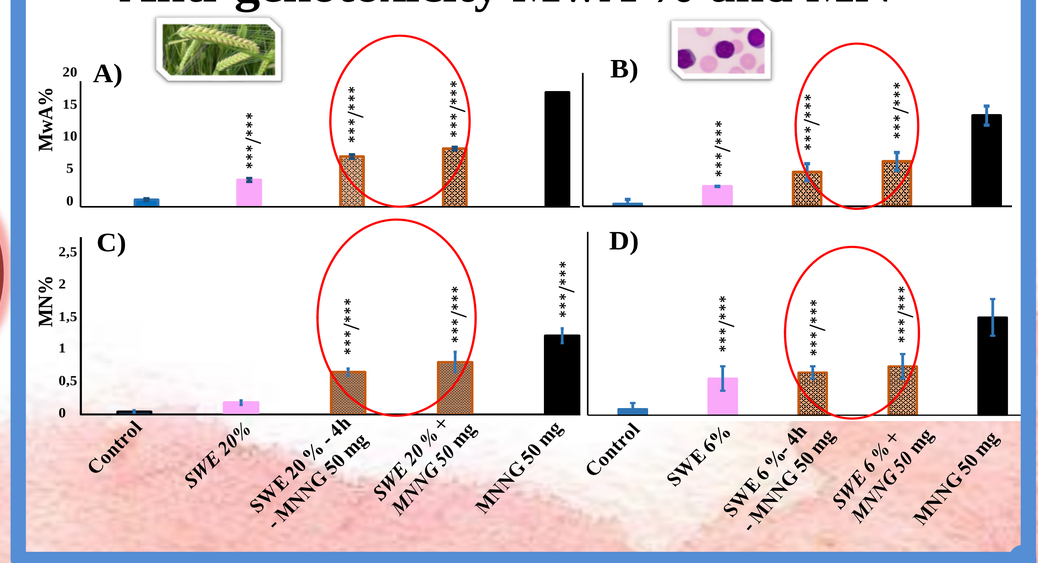

Figure 3. Anti-genotoxic effect of *R. alba L.* SWE assessed by value of chromosome aberrations A), B), and micronuclei C), D) after co- treatment with non-toxic concentrations of extracts and damaged concentration of MNNG with or without inter-treatment time in *H. vulgare* and human lymphocytes *in vitro*, ***p<0.001, before slash-compared to control, after slash –compared to MNNG.

DISCUSSION

The discussion of these findings suggests that the high concentration of monoterpene alcohols and phenolic compounds in the SWE plays a key role in neutralizing the alkylating action of MNNG. The observed cytoprotection/genoprotection in both conditioning and co-treatment schemes indicates that the extract may act through multiple mechanisms: both as a direct desmutagen and by potentially stimulating cellular repair processes.

CONCLUSIONS

R. alba L. flowers SWE exhibits potent anti-cytotoxic and anti-genotoxic activity in diverse cell types. These findings reveal that SWE is not only a promising green technology for industrial production but also a source of high-quality extracts with robust defense potential against DNA-damaging agents.

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