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ASSESSMENT OF CYTOTOXICITY AND GENOTOXICITY POTENTIAL OF WASTEWATER AS A DISTILLATION BY-PRODUCT OF *ROSA DAMASCENA* MILL. ESSENTIAL OIL

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Assessment of cytotoxicity and genotoxicity potential of wastewater as a distillation by-product of *Rosa damascena* Mill. essential oil

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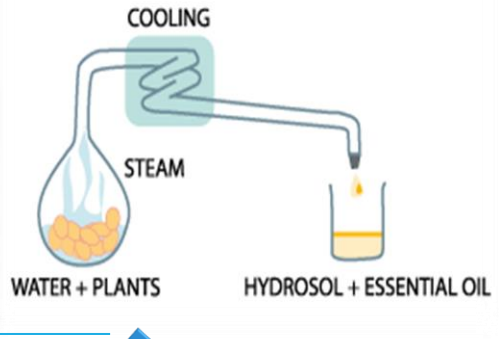

Photo: https://www.rozata.com/blog/rozovo-masla-prilozenie/rosa-damascena/

wastewater

Rose wastewater (WW) is rich in water-soluble compounds - tannins, flavonoids, and total polyphenols of 7.80 ± 0.22 mg/mL (Georgieva et al., 2021)

The liquid residue – **wastewater**, would pose an environmental issue as a pollutant

Limited studies exist about the **cytotoxic and genotoxic activity** of rose **wastewaters** obtained after water-steam distillation of ***rose*** oil.

Insufficient data on the possible genotoxicity of ***R. damascena*** **wastewater** in the literature.

Objective

Evaluate **cytotoxic** ⇅ potential **genotoxic**

to determine the sensitivity of the experimental test system ICR mice ***in vivo*** to ***R. damascena*** wastewater

To accomplish the Aim, a complex of classical cytogenetic endpoints was applied:

- **mitotic index as a cytotoxicity endpoint;**
- **chromosomal aberrations and micronuclei induction - for genotoxicity;**

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MATERIALS AND METHODS

Relevant endpoints for cytotoxicity - mitotic index (MI) and genotoxicity - chromosome aberrations (CA) and micronuclei (MN)

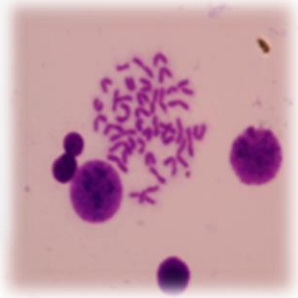
Cytotoxic/genotoxic endpoints

Mitotic index (MI)

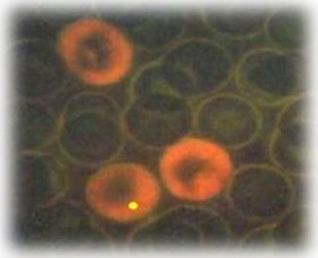
PCE/(PCE+NCE)

PCE - polychromatic erythrocytes
NCE - normochromatic erythrocytes

Chromosomal aberrations (CA)



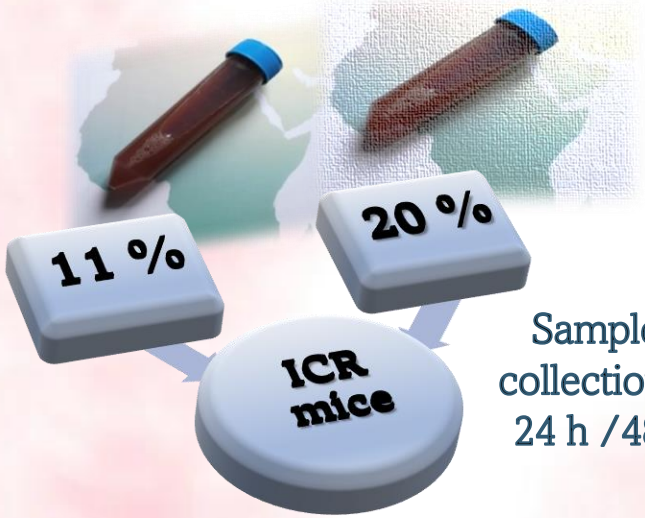
Micronuclei (MN)



TEST SYSTEM USED

Eight-week-old ♂ and ♀ ICR strain laboratory mice *in vivo*

Rosa damascena ww solution concentrations applied:



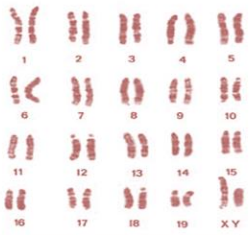
Samples collection at 24 h /48 h

Positive control
N-methyl-N'-nitro-N-nitrosoguanidine MNNG (50 µg/mL)

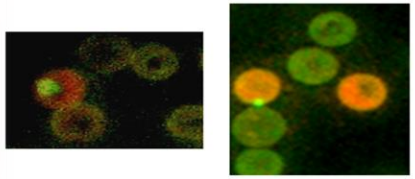
Negative control
NaCl 0,9 %

male and female ICR strain albino mice

mouse karyotype



Micronucleated polychromatic erythrocyte (MNPCE)

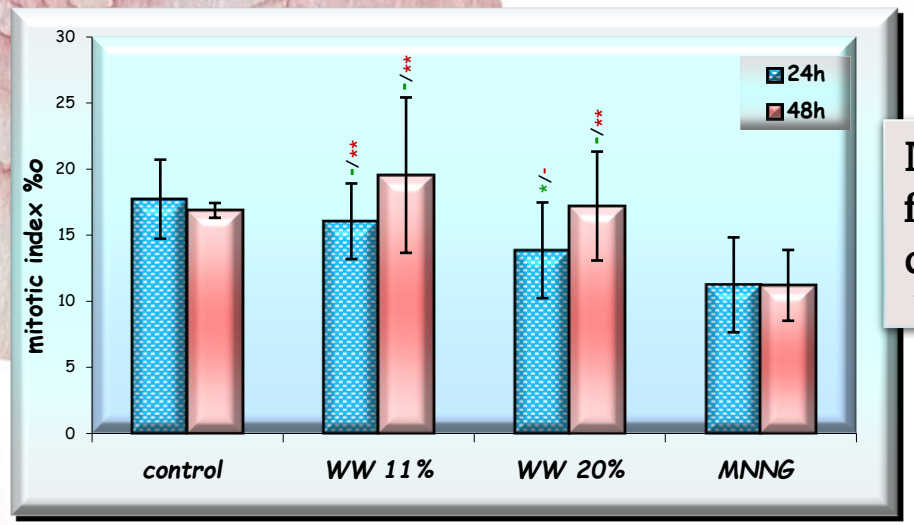


Chromatid aberrations

isochromatid break

RESULTS - Cytotoxicity

Mitotic index (MI)



(MI=A/1000, A-number of dividing cells)

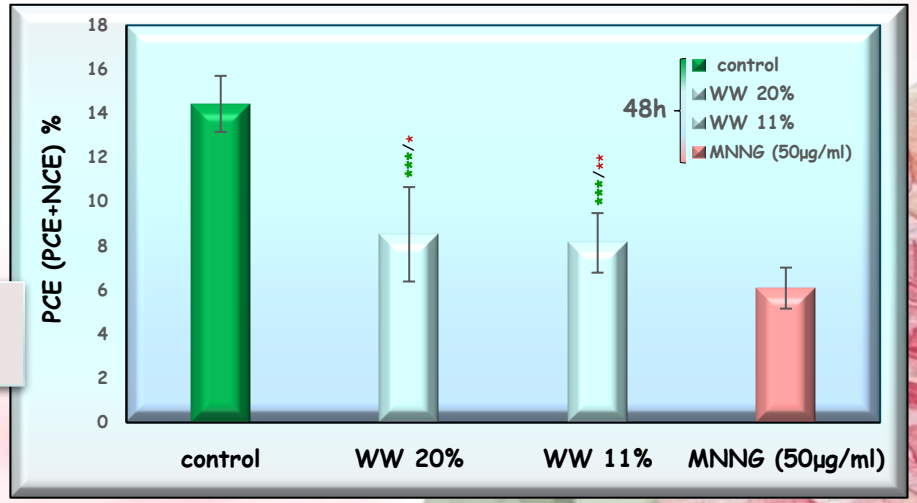
Mitotic activity observed in the bone marrow cells of ICR mice following the application of *R. damascena* ww at two concentrations: an 11% or a 20% solution

*p<0.05, ** p<0.01, (-) not significant; before slash - compared with the negative control, after slash - compared with MNNG

All data are expressed as Mean ± SD

PCE (PCE + NCE)

The ratio of PCE to total erythrocytes scored (PCE+NCE) » an index of cytotoxicity. For each animal, this ratio was determined by counting at least 2000 normochromatic erythrocytes (NCE) per animal



Cytotoxic activity of ww assessed by the PCE/(PCE+NCE) ratio

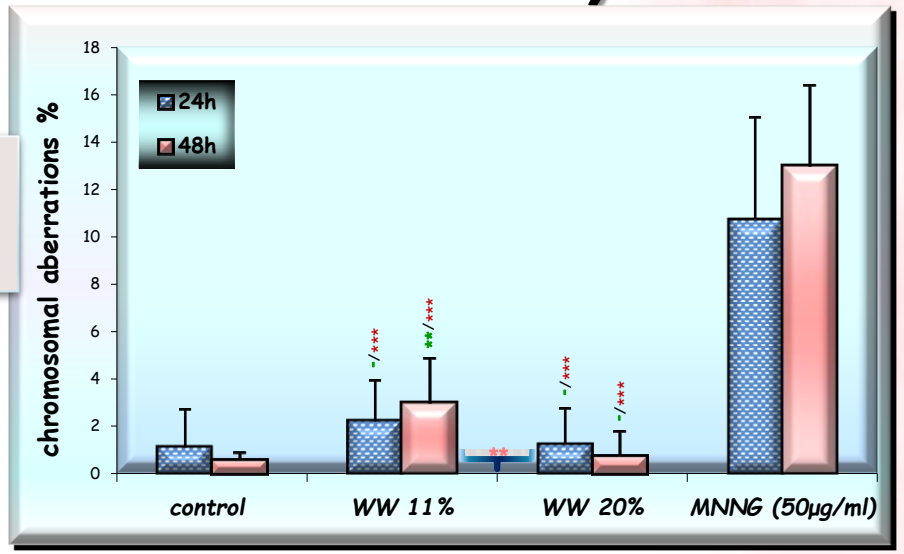
*p<0.05, ** p<0.01, (-) not significant; before slash - compared with the negative control, after slash - compared with MNNG

RESULTS - Genotoxic activity

CA (metaphases with chromosomal aberrations)

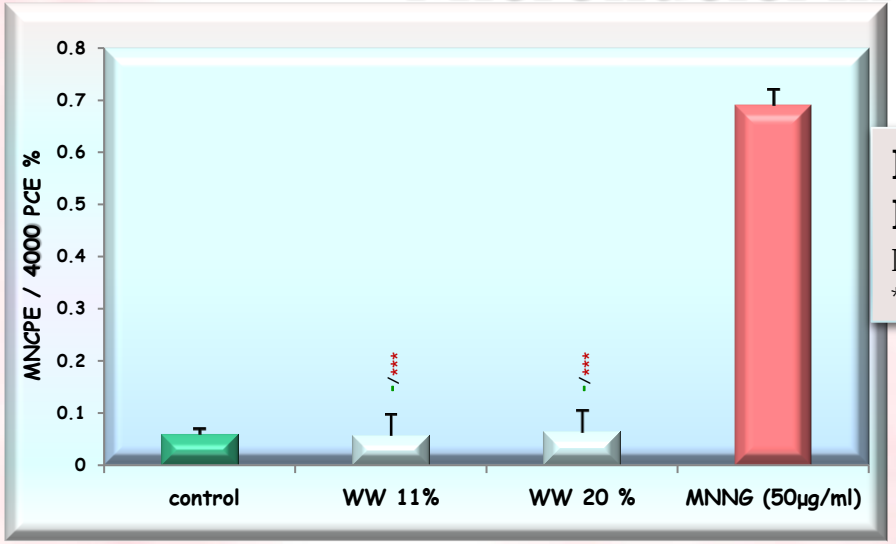
Frequency of chromosomal aberrations (CA), obtained 24/48 hours after a single intraperitoneal supplementation of *R. damascena* ww solution

*p<0.05, ** p<0.01, (-) not significant; before slash - compared with the negative control, after slash - compared with MNNG



A dose-dependent effect (**p < 0.01) was observed between the 20% and 11% solutions.

Micronuclei induction (MN)



Induction of MN observed in the peripheral blood erythrocytes (PCE) of ICR mice 48 hours after a single treatment with *R. damascena* ww solution

MNPCE micronucleated immature erythrocytes

*p<0.05, ** p<0.01, (-) not significant; before slash - compared with the negative control, after slash - compared with MNNG

All data are expressed as Mean ± SD

A slight decrease in cell proliferation (MI assay) was observed solely in the mammalian bone marrow exposed to 20% wastewater for 24 hours ($p<0.05$)

The significant reduction in the PCE/total erythrocytes ratio in the peripheral blood of animals treated with *R. damascena* wastewater indicated **cytotoxicity**

No **genotoxicity** was detected in the animal tests evaluated by CA and MN assays, except for the CA assay at 11% / 48h, which showed a significant increase in CA frequency compared to the negative control ($p <0.01$)

A dose-dependent **genotoxic** effect ($p < 0.01$) was observed between the 20% and 11% solutions (CA assay)

CONCLUSION

The wastewater solution from *Rosa damascena* Mill., at both tested concentrations, did not show genotoxic effects. However, it did reveal a slight to moderate reduction in the proliferative activity of somatic mammalian cells. This may provide a foundation for future research and appropriate methods for valorizing and practically applying wastewaters.

THANK YOU FOR YOUR ATTENTION

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(granted to Prof. Milka Mileva, PhD)