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DNA protective potential of biologically active natural compounds with plant origin

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SUMMARY

Relevance of the problem: Nowadays, the anthropogenic pollution could be considered as one of the main problems leading to high risk of damage at various levels of organization of biological systems. Damages at cellular/subcellular level and especially any changes in genetic material could be regarded as the most important.

One promising strategy for overcoming the negative effects of environmental genotoxins including those with carcinogenic properties, is antimutagenesis. The identification of nontoxic biologically active natural or newly synthesized compounds with antioxidant, DNA protective, antigenotoxic, antimutagenic and anticarcinogenic effects as well as study on the cellular and molecular mechanisms of their action (Nikolić et al., 2012) is in a focus of scientific investigations.

Recently, a lot of attention is paid on the natural products with plant origin for pharmacological application (Kopaskova et al., 2011; Ncube et al., 2012; Isbilir и Sagiroglu, 2012; Vieira et al., 2012; Cragg and Newman, 2013). The worldwide distribution and use in folk medicine make them interesting for the antimutagenesis and genome protection.

The corn poppy (*Papaver rhoeas* L.) and the wild basil (*Clinopodium vulgare* L.) are commonly used in folk medicine because of their anti-inflammatory properties. Based on this knowledge **we have formulated our hypothesis**, according to extracts of *Papaver rhoeas* L. and *Clinopodium vulgare* L. would probably possess antioxidant, DNA protective, antigenotoxic, antimutagenic and anticarcinogenic potential.

Two test systems are used to check this hypothesis – *Saccharomyces cerevisiae* and *Chlamydomonas reinhardtii*.

Saccharomyces cerevisiae are widely used test-system for studying oxidative stress and its related consequences. Results obtained on *S. cerevisiae* could be easily extrapolated at mammalian, including human level because of homology in genes and conservative functions of proteins (Foury, 1997; Hartwell, 2004; Wright et al., 2014).

Chlamydomonas reinhardtii is a robust model for studying genotoxic/antigenotoxic potential of different compounds because of the homology with higher plants and human.

Both test systems are characterized with short life cycle, not requiring expensive consumables and equipment for cultivation.

Main results: It is found that the radiomimetic zeocin possesses pro-oxidant, mutagenic and carcinogenic effects in a test-system *Saccharomyces cerevisiae*.

No any concentration of aqueous capsule *Papaver rhoeas* L. extract (0.25-5mg/ml) acts as a pro-oxidant. For the aqueous leaves' *Clinopodium vulgare* extract pro-oxidant effect

depends on the concentration. The only concentration with pro-oxidant capacity (1000 µg/ml) leading to around 1.5-fold higher level of superoxide anions. The antioxidant capacity of *Papaver rhoeas* L. and *Clinopodium vulgare* L. extracts is confirmed with chemical and biological tests in *Saccharomyces cerevisiae*. Our finding show that antioxidant activity of *Clinopodium vulgare* L. extracts depends on the plant origin and the extraction procedure.

No DNA damaging, genotoxic, mutagenic and carcinogenic effect is found for the *Papaver rhoeas* L. and *Clinopodium vulgare* L. extracts in the tested concentrations' range. Dose-dependent DNA protective, antigenotoxic, antimutagenic and anticarcinogenic effect is revealed for both extracts (*Papaver rhoeas* L. and *Clinopodium vulgare* L.).

For the first time in *Saccharomyces cerevisiae* is clarified the meaning of the term "priming" dose that can induce adaptive response: the dose used must be in the range of LD_{20} - LD_{37}, increasing the yield of reactive oxygen species and/or double-strand breaks around 1.5-fold without any mutagenic and carcinogenic events.

Kaempferol and jatropham protect *Chlamydomonas reinhardtii* DNA via acceleration of DSB rejoining depending on the experimental design.

Contribution: Data obtained would be useful in a future for better understanding of mechanisms involved in antimutagenesis, genome protection and possible application of natural products in pharmacology and could be split in three groups.

New data:

- Antioxidant, DNA protective, antigenotoxic, antimutagenic and anticarcinogenic properties of aqueous extracts of *Clinopodium vulgare* L. leaves and *Papaver rhoeas* L. capsule in *Saccharomyces cerevisiae*.
- Kaempferol and jatropham possess DNA protective properties via acceleration of double-strand breaks rejoining in *Chlamydomonas reinhardtii*.
- Triggering event for the formation of adaptive response in *Saccharomyces cerevisiae* could be around 1.5-fold higher levels of reactive oxygen species that are not accompanied with mutagenic and carcinogenic changes.

Confirmation data:

The "priming" dose required for the formation of zeocin-induced adaptive response in *Saccharomyces cerevisiae* – it should lead to at least 1.5-fold higher double strand break levels than those in the control untreated cells.

Applied data:

CFGE protocol is optimized for *Saccharomyces cerevisiae*.

The optimal experimental conditions for the formation of adaptive response in *Saccharomyces cerevisiae* towards to the radiomimetic zeocin are found: treatment of cells in the beginning of stationary phase; "priming" dose corresponding to LD_{20} - LD_{37} that increases 1.5-fold ROS and/or DSBs levels; 45 min intertreatment time; treatment with test dose higher than LD_{50} and 30 min recovery time.