

*Short communication*

## **Silybin and silydianin diminish the oxidative metabolism of human polymorphonuclear neutrophils**

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**Silybin and silydianin exerted an inhibitory effect on superoxide radical production, peak chemiluminescence and hydrogen peroxide production in human polymorphonuclear neutrophils activated with opsonized zymosan.**

Flavonoids are widely distributed in vascular plants and approximately 2000 individual chemical species of this class have been described. Large amounts of flavonoids are consumed in dietary fruits and vegetables; they are also present in many of traditional herbal medicines. The flavonoids are considered nontoxic. They have been reported to exert multiple biological effects, e.g. protection from viruses and germs, immunomodulation, antioxidation, metal chelation, and free radicals scavenging [1, 2]. Although their beneficiary action has been widely recognized, some hydroxyflavones (quercetin and kaempferol) have been reported to exhibit mutagenicity. Distinct activities of various flavonoids have been thoroughly reviewed [3, 4].

Silybin and its isomer, silydianin are active components of the thistle *Silybum marianum* (L) Gaertn., a component of many po-

pular pharmaceutical preparations: Silymarol, Silycynar, Legalon, etc. Their antioxidant activity has been suggested previously [2, 5, 6]. The aim of our study was to find if the two isomers affect the oxidative metabolism of polymorphonuclear neutrophils (PMN), the phagocytic cells which defend a host from the microbial invasion. Their action is connected with increased oxygen consumption, called an "oxidative burst". Microbial cells activate the NADPH-dependent oxidase, which generates the superoxide radical, which is then decomposed to H<sub>2</sub>O<sub>2</sub> by the enzyme superoxide dismutase, or reacts with myeloperoxidase to form hypochlorous acid. Hydrogen peroxide, superoxide radical and hypochlorous acid exhibit direct antimicrobial activity, but also damage host's tissues and oxidize physiological substrates of crucial biological significance (membrane lipids, nucleic acids and proteins) [7].

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**Abbreviation:** PMN, polymorphonuclear neutrophils.

The data presented below contribute to the experimental evaluation of the favourable action of *S. marianum* preparations.

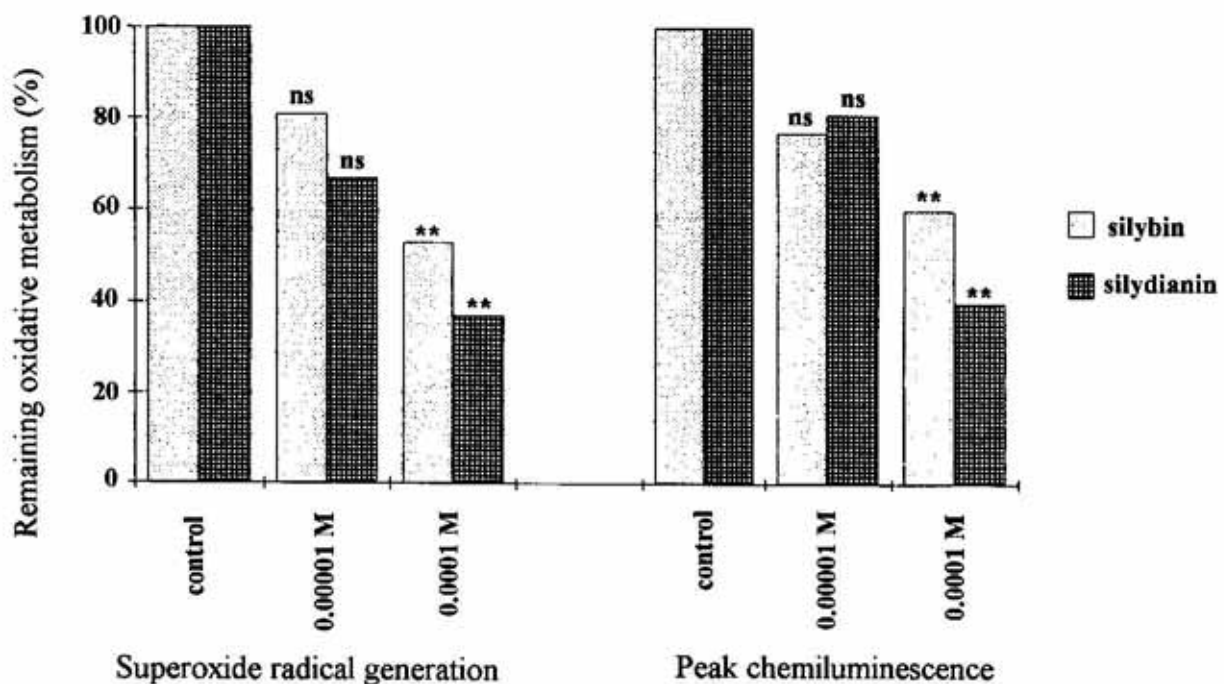
## MATERIALS AND METHODS

Human polymorphonuclear neutrophils were isolated from heparinized blood of healthy donors and volunteers by Gradisol G (Polfa, Kutno, Poland) density gradient centrifugation [8]. Determination of superoxide radical was performed according to Pawlicki *et al.* [9]. We adapted the method to the isolated PMN. The cells were pretreated with  $10^{-4}$  M and  $10^{-5}$  M silybin or silydianin for 10 min at 37°C. Determination of hydrogen peroxide was performed according to Pick &

molecule;  $10^6$  cells were pretreated with  $10^{-4}$  M and  $10^{-5}$  M silybin or silydianin and activated with the opsonized zymosan, the *in vitro* equivalent of a microbial cell.

## RESULTS AND DISCUSSION

Our results show that silybin and its isomer, silydianin, generally diminish the oxidative metabolism of PMN. The superoxide radical generation in stimulated PMN was significantly diminished after incubation with  $10^{-4}$  M silybin or silydianin, however the latter gave a more marked depression (37% of remaining activity *vs* 53%). At  $10^{-5}$  M concentration the two examined compounds caused an only insignificant reduc-



**Figure 1.** Effect of silybin and silydianin on the superoxide radical generation (left) and the peak chemiluminescent signal (right).

The results presented are expressed in per cent of activity remaining after incubation with silybin or silydianin. The control samples (without flavonoids) were considered 100%. Mean values and standard deviations calculated from 12 experiments were used in statistical evaluations: ns = non significant, \*\* =  $P < 0.01$ .

Keisari [10]. The concentrations of silybin and silydianin used for the analysis were the same as above. Chemiluminescence quenching was measured according to Cheung *et al.* [11] using a home made luminometer and luminol as an oxidizable and luminiferous

tion both in superoxide radical generation (Fig. 1) and in chemiluminescent signal. Silydianin at  $10^{-4}$  M concentration exerted a stronger inhibitory effect than did silybin. The hydrogen peroxide production was examined only in the case of silybin, and a trend

towards a dose-dependent decrease was observed (88% for  $10^{-4}$  M and 95% for  $10^{-5}$  M concentration) (not shown).

The observed inhibition of the reactive oxygen species production in polymorphonuclear neutrophils could be extrapolated to other phagocytic cells, and possibly also to other biological reactions in which superoxide radicals and hydrogen peroxide are generated. No toxicity of the *S. marianum* preparations has been reported. The investigation of the beneficiary action of *S. marianum* isolates on the hepatic tissue focussed primarily on their regenerative effects. In experimental liver cirrhosis *S. marianum* reduced both the activity of hepatic enzymes and plasma cholesterol level, and prevented cellular membranes from deleterious peroxidation [5, 6, 12]. Thus the suggested previously antioxidative abilities of silybin and silydianin were confirmed leading to the conclusion that antioxidation is one of several possible mechanisms of the drug action [1, 2]. The properties of silybin were corroborated in many experimental models: it was shown to protect erythrocytes from lipid peroxidation and haemolysis induced by phenylhydrazine and to reduce the oxidative effects of drugs and alcohol on animal and human tissues [1, 12]. The favourable influence of silybin and silydianin on oxidative metabolism might inspire further research on antioxidants of plant origin, which could broaden our knowledge about the mechanisms of the herbal drugs actions. In our department some studies on antioxidative properties of orientin from *Adonis vernalis* (L) were undertaken (submitted). The results obtained are promising, suggesting that the kingdom of plants may supply medicine with yet unknown therapeutic possibilities.

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