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Study the structure-activity relationship of silybin analogues using different ROS production sources

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Reactive oxygen species (ROS) formation is indispensable for life. They play role in optimal working of immune system (killing mechanism), in different signal transduction processes, induction of apoptosis or activation of genes. Elimination of ROS requires presence of enzymatic and non-enzymatic antioxidants, in which natural origin antioxidants from the diet are involved. Overproduction of ROS initiates development of diseases; therefore, antioxidant supplementation was suggested for prevention or treatment of those states. However, results of these studies were contradictory, such as the role of Vitamin E in prevention of cardiovascular diseases which was mainly due to the fact that, in most cases, antioxidant requirement e.g. original Vitamin E level, and source(s) of ROS was not determined or took into account.

To certify the importance of ROS source in antioxidant activity of a given molecule, several silybin (1) analogues were synthesized namely, flavanon- (2, 3), flavone-derivatives (4, 5), flavanolignan skeleton (6), dehydrosilybin (7), and hydnocarpin (8), and their effects were compared in inhibition of superoxide anion production in phorbol-ester stimulated human neutrophils, xanthine oxidase activity, H-donor activity, LDL oxidative resistance, and ferric ion reducing capability.

It was found that only silybin and dehydrosilybin possessed measurable H-donor activity. Ferric ion reduction was observed in case of silybin (0.68 teq), dehydrosilybin (0.45 teq) and hydnocarpin (0.28 teq). Xanthine oxidase activity was inhibited by silybin and its flavanon analogues (2, 3) in similar extent (IC50~32 uM), and flavone analogues of silybin (4, 5), dehydrosilybin and hydnocarpin were more effective (IC50~0.2 uM), and silybin skeleton (6) was absolutely ineffective. Oxidative resistance of LDL increased by 3.2 fold by silybin, 2.7 fold by its flavone analogues, while the other compounds had weaker effects (1.2 fold), and silybin skeleton was ineffective. However, phorbol-ester stimulated superoxide anion production was inhibited most effectively by silybin skeleton (58%), followed by hydnocarpin (52%), dehyrosilybin (50%), and flavone analogues of silybin (40%), respectively. Silybin itself and its flavanon analogues were the less effective (15-22%). The inhibition in superoxide anion production was due to inhibition of PKC-alpha activation in neutrophils. On this basis we can conclude that 1. Inhibition of xanthine oxidase activity requires OH groups, and presence of a double bond in C ring enhances the effect; 2. Ferric ion reduction required the intact flavanolignan structure, and double bond in ring C attenuated the effect; 3. Flavanolignan structure independently the presence of OH groups, and double bond had low H-donor activity; 4. Oxidative resistance of LDL was modified by silybin and double bond in ring C attenuated its effect; 5. However, effective inhibition of PKC in human neutrophils was achieved in case of silybin skeleton, e.g. in the absence of OH groups, 2-methoxy-4 hydroxy-phenyl, and 3-hydroxymethyl groups, in the presence of one or more side chain(s) attenuated the effect of silybin skeleton.

In summary, these results clearly show that relationship between structure and antioxidant capacity of a given antioxidant is depend strongly on type(s) of ROS source. Therefore, we suggest the identification of ROS source before initiation of antioxidant supplementation therapy.

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