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A089 Immunomodulatory effects of the methanolic extract of *Epimedium alpinum* L.

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Epimedium species have been shown as traditional Chinese herbal medicine widely used in treatment of rheumatic diseases, nephritis, hepatitis C and hematological consequences of anticancer therapy. Certain flavonoid components such as epimedin C, icarin or baohuosides isolated from *Epimedium* species posses immunomodulatory (enhancing or suppressive) activities (1). Genus *Epimedium* in Serbia is represented by only one species, *Epimedium alpinum* L. The data on constituents of this species are very poor. In this work the effect of methanolic extract of root and rhizome of *E. alpinum* (MEEA) on proliferation of rat lymphocytes *in vivo* and *in vitro* was studied.
We first showed that the extract in concentrations ranging between 10 µg/ml and 250 µg/ml inhibited proliferation of thymic and splenic lymphocytes *in vitro* in a dose dependent manner triggered by Concanavalin A (ConA), a potent T-cell mitogen (2). The effect correlated with decreased production of IL-2 and was a consequence of induced apoptosis (programed cell death) of lymphocytes. In some experiments lower concentrations (usually 0,1 µg/ml) of MEEA stimulated proliferation of splenocytes in the presence of ConA and the effect correlated with increased NO production. When rats were immunized in foot pad with Keyhole limpet hemocyanine (KLH), emulsified in complete Freund adjuvans, with or without MEEA (1 mg), a significant increase in cellularity of popliteal dreaning lymph node (LN) in MEEA treated animals was observed 9 days later. In addition, a significant stimulatory effect of LN lymphocytes in MEEA treated rats was achieved *in vitro* after addition of KLH. Lower concentrations of MEEA (0,1 µg/ml) also significantly stimulated proliferation of LN lymphocytes in the presence of R73 monoclonal antibody, recognizing the rat $\alpha\beta$ T cell receptor. However, when MEEA was applied *in vitro* together with KLH only suppressive, dose dependent, effect on LN lymphocyte proliferation was demonstrated. These results show that the extract of *E. alpinum* also posses immunomodulatory activity, suggesting that certain active components may be further studied as immunotherapeutic agents.

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A090 eNOS inhibition by 3,5-nonadiyne, the main constituent of *Cachrys ferulacea* (L.) Calestani essential oil

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Cachrys ferulacea (L.) Calestani syn. *Prangos ferulacea* (L.) Lindley (Apiaceae) is a plant widely distributed in Mediterranean region, as well as at Caucasus, in Turkey, Iraq, Iran and India. In Serbia, this taxon is supposed to be critically endangered. It is used at Caucasus as salad and in Turkish folk medicine as tonic, antiflatulent, for intestinal worms, wounds and external bleeding (1). Manunta (2) suggests that *C. ferulacea* is botanically identical to old plant Silphion (*Silphium*), indicating its antique interest. The chemical analysis of *C. ferulacea* collected from natural population of Montenegro is a part of our study of domestic flora. The root contained 0.4-1.2 % of essential oil. GC and GC/MS analyses indicated 3,5-nonadiyne as a main component of this oil. 3,5-nonadiyne was isolated by preparative gas chromatography and unambiguously spectrometricaly (MS, CIMS, IR, UV, ^{1}H-NMR, ^{13}C-NMR, DEPT, 2D-NMR HH-COSY and HETCOR) identified. The biologically relevant activity of 3,5-nonadiyne was evaluated by measuring its effect on macrophage nitric oxide (NO) production and T-cell proliferation. Concentration dependent inhibition of endogenous NO production revealed inhibitory concentration (IC_{50}) of 6.7 µM. LPS stimulated production of NO was inhibited slightly, by 100-1000 times higher concentration. Basal NO production by macrophages may be due to signalling and regulating effect of NO. Elimination of basal emission of NO by 3,5-nonadiyne does not interfere with LPS stimulated NO production in macrophages. According to a (3) basal (endogenous) level of NO production and concentration have influence on peak concentration of iNOS generated NO. That has implication on severe inflammation processes. Mitogen lectin-stimulated rat T lymphocyte proliferation was employed for evaluating immunotoxic activity of 3,5-nonadiyne. Results showed that this compound does not inhibit rat T lymphocyte proliferation, indicating low toxicity.

References: 1. Baser, K. et al. (2000) Planta Med., 66: 346-347. **2.** Manunta, A. (1996) Studio miscellanei Edit. L'erma di Bretschneider, 29: 211-218. **3.** Connelly, L. et al. (2001) The Journal of Immunology, 166: 3873-3881.