A107 *In vitro* antigenotoxic effect of *Rhus aromatica* root bark

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For the prevention of the DNA damage, natural products have already been taken a significant role using their antioxidant potentialities. The present study deals with the investigation of the potential protective properties of *Rhus aromatica* extract and fraction against H_2O_2 induced toxicity in human lymphocytes by comet assay (single cell gel electrophoresis; horizontal electrophoretic chamber, LMP-agarose 1%, electrophoreses buffer; 20 min, 25 V; cells stained with ethidium bromide). *Rhus aromatica* Ait (Sumach, Anacardiaceae) is widely distributed in North America, East Asia and South Africa. Literature reports about antiviral (1), antibacterial and antioxidant (2) activities. The herbal drug is used for the treatment of disturbances of the urinary tract. Fatty acids, triterpens, sterols, tannins, essential oil and flavonoids were proved in the root bark (3). The powdered drug was extracted with 30% EtOH under reflux (extract), another drug part was extracted with 30% EtOH after separation of the essential oil (fraction). The isolated human peripheral lymphocytes were treated with the extract and the fraction in conc. of 5-100 µg/mL. The viability of the cells was unaffected (>90%) at the concentration tested. Using tail length (µm) as quantitative parameter for the comet formation it was observed that the 30% EtOH extract did not cause any strand breaks up to 100 µg/mL (N=3; mean value 7.22 µm) while the fraction caused DNA damage at 80 and 100 µg/mL (N=3; mean value 42.27 and 38.96 µm, resp.) when compared with untreated control (N=3; mean value 4.22 µm). It can be concluded that the extract and the fraction of *Rhus aromatica* are non-toxic to lymphocytic cells *in vitro*. In a further experiment the protective effect of the drug was investigated. Therefore the cells were pretreated with extract and fraction (conc. 5-40 µg/mL) and subsequently exposed to 75 µM H_2O_2. The cells exhibited a significant dose-activity relation in the reduction of DNA strand breaks. The protective effect of the extract and the fraction on lymphocytes damaged by H_2O_2 is significant.

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A108 Inhibition of metalloproteinase-9 activity and gene expression by polyphenolic compounds isolated from the bark of *Tristaniopsis calobuxus* (Myrtaceae)

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Metalloproteinases (MMPs) play an important role in pathological conditions including tumor metastasis, periodontitis, osteoarthritis, chronic ulcerations and contribute to the atherosclerotic plaque fissuration, leading to atherosclerosis complications (1). The methanolic extract of *Tristaniopsis calobuxus* bark was shown to inhibit elastase (2) and plasmepsin II (3) activities, therefore the extract was tested on the activity of metalloproteinase-9 (MMP-9) in cultured macrophages. The methanolic extract (10-25-50 µg/ml) dose-dependently reduced (-30%, -65% and -95%) the activity of MMP-9 secreted by macrophages. After fractionation of the crude extract, the inhibitory activity was retained in the ethyl acetate fraction (50%, 75%, and 95% inhibition). Treatment of the cells for 24 hours with the ethyl acetate fraction (10-50 µg/ml) significantly reduced the release of MMP-9, up to 80%. No appreciable cellular toxicity was observed, even at the highest concentration used. The ethyl acetate extract was chromatographed on Sephadex LH 20, obtaining 7 fractions from which pure phenolic compounds were isolated. The inhibitory effect on MMP-9 secretion was associated to fractions 5A and 5B and to ellagic acid, while other phenolic compounds were not active (gallic acid, p-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid). To investigate if the effect on protein secretion was related to MMP-9 gene regulation, 5A, 5B and ellagic acid were tested on MMP-9 promoter activity. The tested compounds (1-20 µg/ml) dose-dependently reduced the MMP-9 promoter-driven transcription of the luciferase reporter gene. Preliminary HPLC-MS analysis of 5A and 5B indicated the presence of gallocatechins, ellagic acid and its glycoside derivatives.

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