

compounds, flavonoids and organic acids. The purpose of this work is the comprehensive chemical analysis of these extracts, implying their fractionation, and the evaluation of their structure-activity relationship.

Extracts of lyophilized and powdered *Sempervivum tectorum* leaves with different polarity (water, 80% (v/v) methanol, methanol, ethanol, acetone, ethyl-acetate and chloroform) has been studied by LC-ESI-MS/MS. Towards a more detailed phytochemical analysis extracts prepared with methanol and chloroform have been fractionated by column chromatographic methods, and the fractions have been studied by LC-MS. *In vitro* antioxidant activity of extracts and fractions has been determined by spectrophotometry using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-(3-ethyl-benzothiazolin-6-sulfonic acid) radical scavenging activity assays. Antioxidant activity has been characterized by IC₅₀ values.

The *Sempervivum* leaf extract, prepared by acetonic extraction and acidic hydrolysis had the highest antioxidant capacity. It contained purely flavonoid aglycones and its antioxidant activity was comparable to the one of kaempferol standard. The extract with the second highest antioxidant activity was prepared by 80% (v/v) methanol. Its LC-MS evaluation proved the presence of rutin, kaempferol-di(rhamno)-hexoside and five other kaempferol-glycoside derivatives. Houseleek extracts affected more potent radical scavenging activity against ABTS, than DPPH. Total polyphenolic content and antioxidant activity of *Sempervivum* extracts showed a significant correlation both ABTS and DPPH radicals ($r^2=0,907$ and $r^2=0,967$, respectively).

LC-MS evaluation of *Sempervivum* proved to have a high content of flavonoids and other phenolics, which are assumed to play an important role in the eminent scavenging activity of the extracts. On the basis of correlation between total polyphenol content and antioxidant activity of extracts it was concluded, that the remarkable scavenger activity of *Sempervivum* is due to the synergism of its polyphenolic compounds.

Reducing oxidative stress and leukocyte activation in reperfusion injury with controlled reperfusion

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Reperfusion of the limbs after acute and persistent ischaemia is associated with high rates of morbidity and mortality despite complete revascularisation. Reconstruction of blood flow will induce reperfusion injury with oxidative stress and inflammatory responses. There are experimental evidences that modification of the initial reperfusion modalities can minimize this reperfusion injury.

In our study we aimed to confirm in an animal model that controlled reperfusion (CR) can reduce oxidative stress and leukocyte activation in reperfusion injury.

In our work we used 10 yorkshire pigs that were divided in two groups. All of the animals underwent a 4 hours infrarenal aortic occlusion: after anesthesia we made median laparotomy and clamped the infrarenal abdominal aortae. In the first group after occlusion we removed the clamp, restored the blood flow and closed the wound. In the second group after ischaemia we made CR. CR consisted of 30-minute infusion of a crystalloid reperfusion solution that was mixed with oxygenated blood (the blood:reperfusion solution ratio was 1:1) distal to the occlusion. After this procedure we restored the normal blood reperfusion.

Blood samples were collected before occlusion, on the end of ischaemic period, and after reperfusion in the 15th minute (from inferior caval vein), in the 1st and 24th hour, and on 7th day (from peripheral vein). To monitor the evoked oxidative stress superoxide-dismutase activity and reduced glutathion concentration were measured. The degree of lipidperoxidation was marked with the quantity of malondialdehyde. The inflammatory response was marked with the measurement of leukocyte activation. PMA induced free radical production of the leukocytes was measured.

The lipidperoxidation was significantly lower in the early reperfusion in the CR group. CR also led to a smaller depletion of the antioxidant enzymes. The speed and rate of free radical production of leukocytes were significantly lower in CR group ($p<0,05$).

The results from this study strongly suggest the hypothesis that the results of conventional embolectomy for acute, severe lower-limb ischemia can be improved by CR. The study was supported by OTKA- K67731.