Anti-inflammation assays

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The aim was to establish effective systems to measure the anti-inflammatory potential of ethnopharmacologically used substances. To avoid animal-experiments a modified HET-CAM-Assay (Hens-Egg-Test at the Chorion-Allantoin-Membane), developed by Theisen and Luepke (0,1) is employed.

The second assay is targeted to a special enzyme system connected to inflammation, which measures lipoxygenase-activity via HPLC (2).

Anti-inflammatory activity of *Harpargophytum procumbens* has been demonstrated in different experiments in rats (3,4,5). In vitro experiments showed that the iridoid glycoside harpagoside inhibits the cyclooxygenase- as well as the lipoxygenasepathway of the arachidonic acid cascade (7). Therefore we have tested extracts of *Harpagophytum procumbens* in the modified HET and the LOX-enzyme-assay.

The plant was extracted three times to give a 60%-, 30%-, and 0%-ethanolic extract, dried and weighed. Its components are analysed and quantified via HPLC (RP-18 column, gradient elution, UV-detection at 280nm, external standart, after (6)).

Het-cam assay modified

Fresh fertile White Legorn eggs (Lohmann Selected Leghorn, LSL) were inoculated on day 9 with the substance supposed to have an antiinflammatory effect. A blank control and a positive (Aspisol) were injected as well. An average of 6 eggs per substance was taken. After further incubation for two hours the eggshell above the cell is removed and the CAM (Chorion-Allantois-Membrane) is exposed.

After dropping 0.3 ml SDS (sodiumdodecylsulfate) on the CAM, time is measured for certain reactions to appear. These were
1.) injection of blood into the vessels; 2.) haemorragia and 3.) A prolongation period of these events is indicative of anti-inflammatory effect.

All extracts showed antiinflammatory activity in low concentrations down to 0.001 mg/egg.

The 30%-ethanolic extract of *Harpargophytum procumbens* turned out to be slightly more potent than the 60%- followed by the 0%-ethanolic extract. (Figure 1)

Lipoxygenase assay

Leucocytes were isolated from human Buffy-Coat blood. After addition of the testsubstances the reaction is started by incubating them at 37°C with arachidonic acid and Calcium-Ionophore. Each sample contains around 106 Leucocytes.
The products LTB4, 15- 12- and 5-HETEs were measured in an isocratic HPLC-System using a mixture of water and methanol as solvent-system.
The hight of the peaks also gives an indication of quantitative effects. Peak areas of the products reflected the degree of enzyme inhibition.
In this Assay-system the 60%-ethanolic extract seems to be the most potent, followed by the 30 %- and then the 0%-extract. (Figure 2)

Conclusion

Aqueous and hydroalcoholic extracts of *Harpargophytum procumbens* exhibits a clear anti-inflammatory effect in our bioassa. A 30%-ethanolic extract is the most potent in the HET-CAM-assay, whereas lipogxygenase activity is inhibited maximally with the 60%-extract.
Considering costs and reproducebility in comparison to in vivo animal experiments HET-CAM-assay and lipoxygenase-assay are suitable systems for testing antiinflammatory activities of all sorts of

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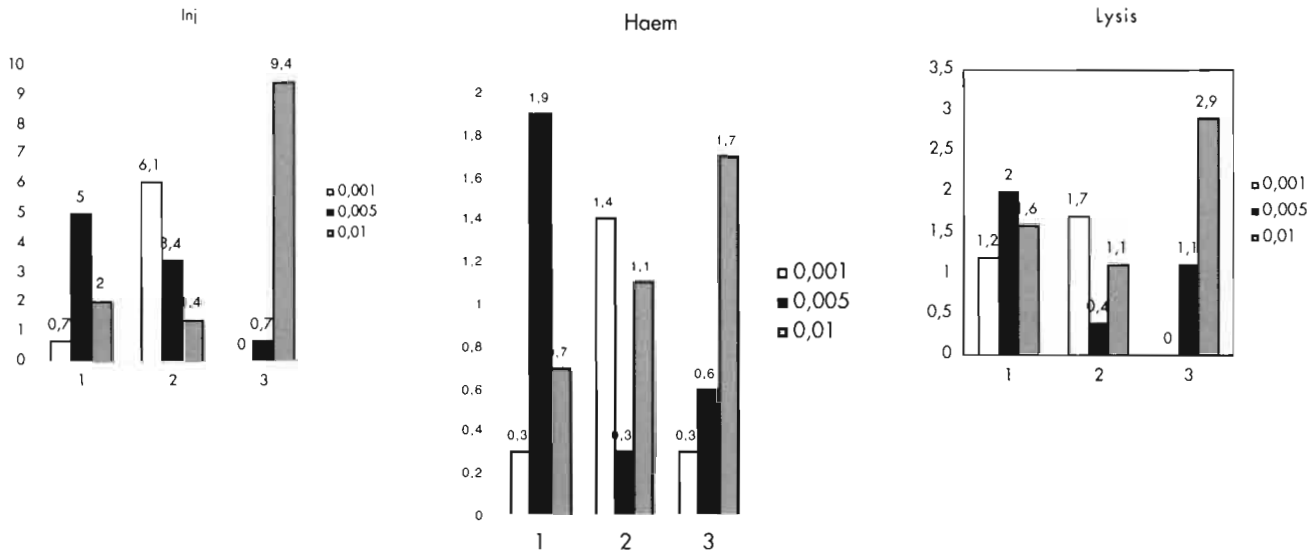

Figure 1. Relative delay of injection/ haemorrhagia/ lysis by 60, 30, 0 % ethanolic extract of *Harpagophytum procumbens*

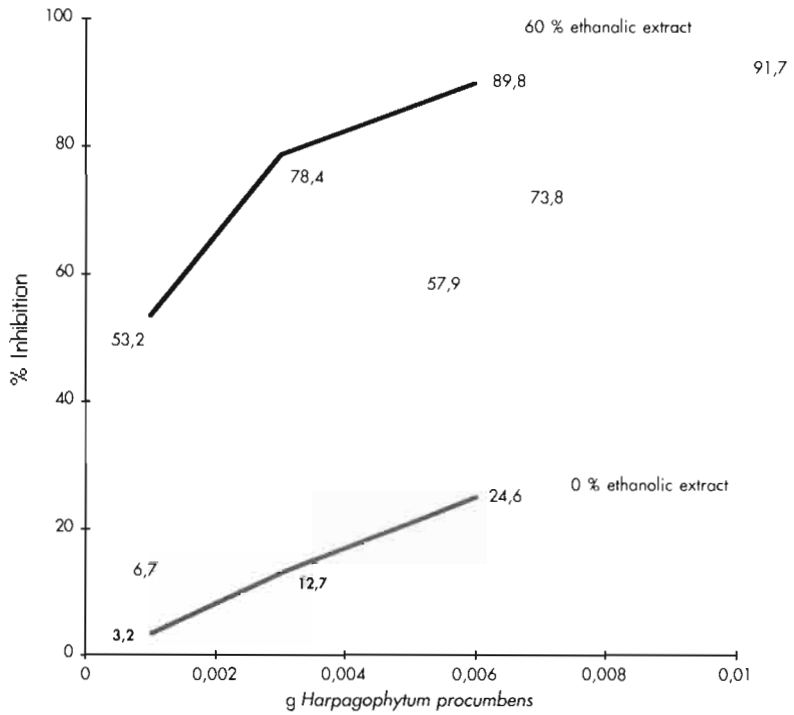

Figure 2. Inhibition of lipoxygenase activity by Harpagophytum procumbens in different extracts of human buffy coat blood