Cytotoxicity and contents of protoanemonin and saponins in pharmaceutical extracts from *Helleborus niger* and *Helleborus foetidus* are different

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Background

Pharmaceutical extracts from *Helleborus niger* L. and *Helleborus foetidus* (L.) MOENCH have become important in complementary medicines, e.g. for supportive treatment of different tumours and haematological malignancies [1]. The observed therapeutic effects are mainly ascribed to saponins [2] but due to its cytotoxic properties, protoanemonin may also be relevant. For *H. niger* but not yet for *H. foetidus* also cytotoxic activity against different tumour cells *in vitro* via induction of apoptosis has been shown [2,3]. The specific composition of extracts from both drugs is determined by the plant material used (species, plant parts, harvest times) as well as the specific production process.

Questions

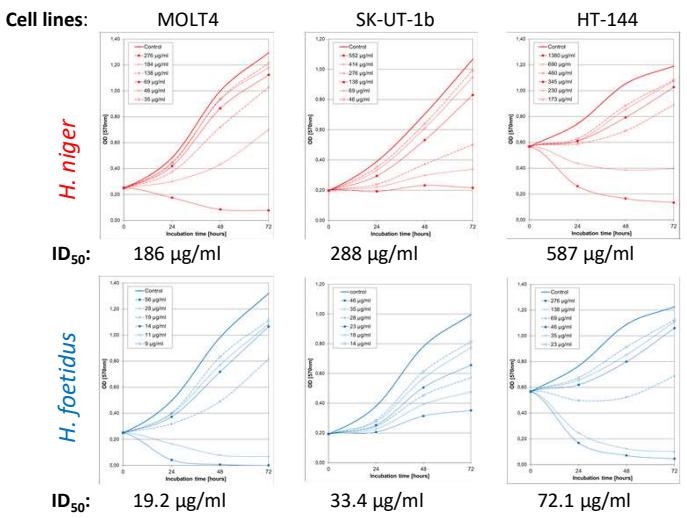
- Investigation and comparison of the cytotoxic and apoptosis-inducing capacities of *H. niger* and *H. foetidus* against tumour cells and human lymphocytes.
- Evaluation of the protoanemonin and saponin concentrations of different organs of both species.
- Impact of two different pharmacopoeial manufacturing methods: Ph. Eur. 1.3.1 (maceration) and GHP regulation no. 34c (fermentation) on the protoanemonin and saponin concentrations.

Materials and Methods

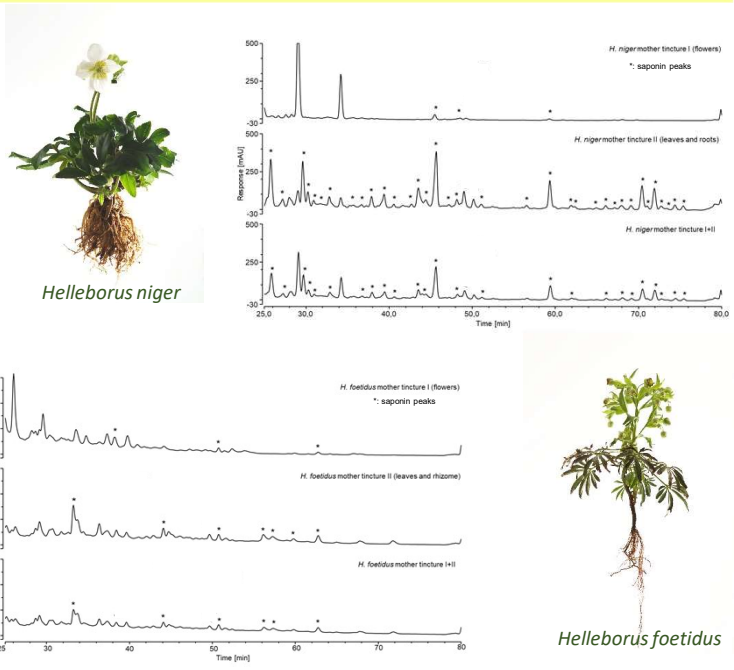
- Extracts from *H. niger* and *H. foetidus* were obtained according to Ph. Eur. 1.3.1 or GHP reg. no. 34c.
- Cytotoxic activity and induction of apoptosis of the extracts were evaluated in the tumour cell lines MOLT4, SK-UT-1b and HT-144 as well as in cultured human lymphocytes isolated via ficoll gradient centrifugation and MACS® cell sorting from venous blood of six healthy donors [4].
- Cell growth of tumour cells was determined via MTT test and apoptosis via flow cytometric analysis of Annexin V-FITC and propidium iodide stained cells.
- Protoanemonin and saponin concentrations were determined by HPLC-DAD [4] and [5].

Results

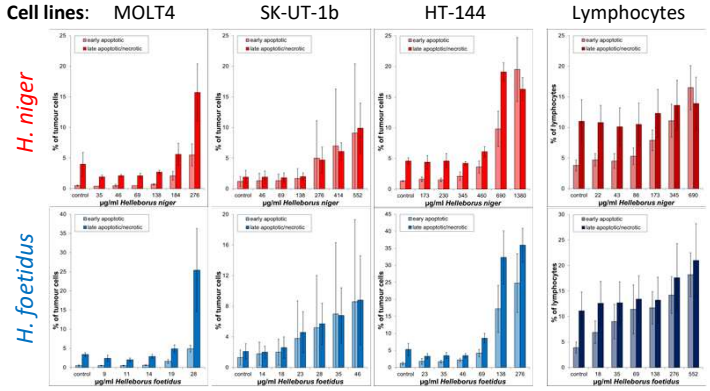
Concentration-dependent growth inhibition of tumour cells



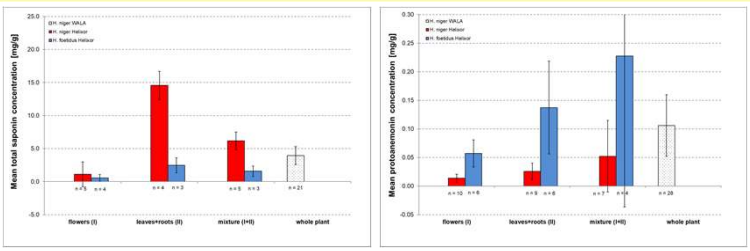
HPLC-DAD-profiles of *H. niger* and *H. foetidus* extracts (Ph. Eur. 1.3.1)



Apoptosis-induction in tumour cells and human lymphocytes after 48 hours of incubation



Mean total saponin and protoanemonin concentrations of *Helleborus* extracts (Ph. Eur. 1.3.1 and GHP reg. no. 34c)



Conclusions

The present investigation demonstrates that *H. foetidus* has similar but significantly stronger cytotoxic and apoptosis-inducing activities than *H. niger*. This may be ascribed to the distinct profiles of saponins and protoanemonin found in the individual *Helleborus* species and to the specific ratio of the plant parts processed.

References

1 Schlodder D. Merkurstab 2010; 63: 500-507
2 Felenda J. et al. BMC CAM 2019; 19: 105-117.
3 Jesse P. et al. Pediatr. Blood Cancer 2009; 52: 464-469
4 Schink M. et al. J Ethnopharmacol 2015; 159: 129–136
5 Müller M. et al. JPBA 2020; 188, 113370

NOTE: A publication containing the data presented here is currently in preparation.