Abstract

Due to the increasing development of resistance of parasitic nematodes to standard anthelmintic drugs, the use of herbal preparations, especially tannin-containing extracts, for the treatment of helminthiasis is widely discussed. The anthelmintic activity of tannins has been demonstrated in various studies both *in vitro* and *in vivo*. However, little is known about the detailed mode of action in nematodes. Within these investiagtions, the mechanisms of anthelmintic action of oligomeric procyanidins from a phytochemically characterized hydroethanolic (1:1 v/v) extract of *Combretum mucronatum* Schumach. & Thonn. leaves (CM) were investigated at ultrastructural and molecular level. For this purpose, the free-living nematode *Caenorhabditis elegans* was used as a model organism for parasitic nematodes.

At the ultrastructural level, the cuticle of *C. elegans* was identified as the main binding site for the tested procyanidins in a concentration of 2 mg/mL using various microscopic methods (DIC, fluorescence- and atomic force microscopy). Underlying tissues showed little changes. After treatment with 2 mg/mL or 0,2 mg/mL CM, significant impairments in the motility of adult worms like a reduction of the wave initiation rate by 32 % after 3 h and 70 % after 24 h, or a reduction of the travel speed by 35 % after 3 h and 84 % after 24 h of treatment with 0.2 mg/mL CM were measured. Furthermore, molting defects in all four larval stages of the nematodes were observed after treatment with 2 mg/mL CM respectively 2 mg/mL isolated Procyanidin C1 (PC1). Force spectroscopic measurements confirmed that interaction of procyanidins with the cuticle increased cuticle stiffness. Besides direct effects of procyanidins binding to the worms' surface proteins, such a change in the biomechanical properties of the cuticle could provide a plausible explanation for various anthelmintic effects described in parasitic nematodes after treatment with tannin-rich extracts. Using fluorescence microscopy procyanidins were observed to accumulate especially in the anterior part of the nematodes. The reduced motility could therefore also be caused by the impairment of amphid neurons located in the head region, disturbing sensomotoric processes of the nematodes.

At the molecular level, the expression and function of the genes *T22D1.2* and *clx-1* which were shown to be strongly upregulated in *C. elegans* after contact with procyanidins, were elucidated in more detail. Both genes were confirmed to be coding for proteins by isolation and mass spectrometric analysis of the respective GFP fusion proteins. Using GFP reporter strains, expression of both genes was shown to be induced quite specifically upon treatment with the procyanidins (and also tested hydrolysable tannins) and not as part of the general stress response. Due to the strong upregulation in response to the treatment and the distinct repetitive proline-rich sequences of both proteins which favour a high affinity for tannins, both proteins were thougt to act as a protective mechanism during tannin exposure. However, the impact of *T22D1.2 and clx-1* on the survival of tannin treated worms was tested on different overexpression and knockout mutants for both genes but was limited to certain mutants and low test concentrations.

Overall, the results of this work provided further evidence regarding potential mechanisms of action of oligomeric procyanidins in *C. elegans*. It can be postulated that the anthelmintic effect is due to various interactions with proteins located at the cuticular surface region of the worm. In addition, procyanidins induce the synthesis of proline-rich proteins in *C. elegans*. The exact function of these proteins has not been fully clarified so far and thus provides an interesting clue for further research.