

New strategies for the treatment of urinary tract infections: Influence of plant extracts from traditional herbal medicine on Tamm-Horsfall Protein

Summary

Urinary tract infections (UTI) are one of the most common bacterial infections, which are often caused by uropathogenic *Escherichia coli* (UPEC). Age, hygiene and sex are relevant for the occurrence of UTI: women suffer from UTI more often than men, and older patients more often than younger ones. The German guideline recommends antibiotic therapy in most cases but due to increasing bacterial resistances against widely used antibiotics and the increasing supply shortages in the context of global crises, alternative treatment options are therefore necessary. The use of herbal medicinal products offers versatile treatment options that are widely accepted by the population. As previously shown, extracts from *Orthosiphon stamineus* leaves or cranberry fruits (from *Vaccinium macrocarpon*) exert anti-adhesive properties against UPEC and therefore represent promising options to the current antibiotic therapy. Another novel strategy against UTI is the stimulation of Tamm-Horsfall Protein which acts as a part of the innate immune defence within the urinary tract: Tamm-Horsfall Protein (syn. Uromodulin, THP) is a highly mannosylated glycoprotein which is produced in the kidney in the thick ascending limb of the Henle loop. N-glycans of THP bind to the mannose-sensitive adhesin FimH of UPEC and thus prevents the FimH-dependent adhesion of bacteria to host cells and facilitates the excretion of pathogens.

To investigate a potential influence of plant extracts on THP stimulation a cost-effective enzyme-linked immunosorbent assay (ELISA) for THP quantification in urine was developed and validated for quantitative monitoring of potential THP changes in urine samples. This ELISA was subsequently used as an analytical tool for the evaluation of THP content in urine samples from volunteers within three different biomedical studies. Furthermore, a protocol based on high resolution mass spectrometry was developed for investigations on the N-glycosylation pattern of THP in urine samples.

In order to investigate gender-specific differences in THP excretion, biomedical studies (identification numbers 2019-177-f-S and 2021-084-f-S) were conducted with a total of 239 volunteers (93 males, 146 females of whom 70 were taking hormonal contraceptives regularly). Female subjects showed higher THP excretion in urine than men ($p < 0.01$). However, women with regular medication with hormonal contraceptives showed reduced THP levels in contrast to women without the regular intake of hormonal contraceptives. Furthermore, the individual N-glycosylation pattern of THP from urine samples from these studies (10 women and 10 men) was investigated. Gender-specific differences were found: Female subjects tended to have an increased proportion of mannose-type glycans and non-fucosylated complex-type glycans, while men showed an increased proportion of fucosylated complex-type glycans.

Herbal medicinal products and the respective preparations that are traditionally used for UTI have been monographed for a variety of plants by the Herbal Medicinal Products Committee (HMPC) of the European Medicines Agency (EMA): The proposed mode of action is described by the HMPC in many cases as being based on an increase in urinary flow to facilitate excretion of UPECs. However, diuretic effects have only been proven in some cases. For this reason, two biomedical studies (2021-084-f-S; 5101/001) were carried out to investigate a potential stimulation of THP by the herbal preparations and, within the same study, to investigate an influence on the electrolyte concentrations in the urine samples in order to identify possible diuretic effects after p.o. intervention with herbal products: *Orthosiphon stamineus* leaves infusion, lovage root infusion, juniper berry infusion, stinging nettle leaf extract, mate leaf extract, dandelion herb extract and horsetail herb extract were administered p.o. for seven days

in the respective study arms, and morning midstream urine was collected at days 0 (untreated control), 3, 6 and 8. A consistently significant increase in THP excretion (up to 400 %) was observed in the *Equisetum* group (daily dose 5.8 g herbal material). Additionally, diuretic effects were monitored for the *Equiseti herba* treated subjects by the sodium and silicon excretion.

Additionally, the pooled urine from the *Equisetum* group exerts significant anti-adhesive effects within an *ex vivo/in vitro* adhesion assay with UPEC NU14 on T24 bladder cells (up to 75 % inhibition). The horsetail extract itself had no anti-adhesive effects in the same test system, indicating the inhibition of UPEC-T24 cell interaction is due to the increased THP concentration and/or the altered composition of the urine due to diuresis after intervention. A significant positive correlation between THP excretion and induced diuresis was found in a multivariate correlation matrix, suggesting that stimulation by THP is accompanied by diuresis (or *vice versa*). In addition, THP N-glycosylation pattern was investigated by mass spectrometry after oral intake of *Equiseti herba* extract (urine samples from day 0 vs day 6; 4 men and 4 women each). No difference in glycosylation was found regarding the whole population, however, in the subgroup analysis of men, there was a significant reduction in fucosylated complex-type N-glycans and a significant reduction in sulphated N-glycans (days 0 vs 6). No relevant changes in N-glycosylation pattern were found in women after oral administration.

Juniper berries showed a mild diuretic effect, and dandelion herb extract showed a diuretic and THP-stimulating effect on day 3. All other plant extracts did not influence the THP excretion.

In addition, the *Equiseti herba* extract was characterised by LC-UV-MS, to revise the analytical methods described for identity and purity testing of this plant by the European Pharmacopeia. The developed LC-MS protocol identified more than 80 % of all eluted peaks and was shown to be able to detect alkaloids such as palustrine and palustridiene, as well as kaempferol-3-O-rutinoside-7-O-sophoroside, which are exclusively described for *E. palustre*, but should be absent in *E. arvense*.

Soluble silica in urine samples and in the extract was subsequently quantified by ICP-OES. A total of ~ 0.06 % (m/m) soluble silica was determined in different *Equiseti herba* extracts, which was in accordance with the requirements of Ph. Eur. 10 for silica content.

The HMPC recommends the use of a freshly brewed lovage root infusion from *Levisticum officinale* for the treatment of UTI, therefore a lovage root infusion (LHI) and an aqueous lovage root dry extract (LWE) were investigated both phytochemically and for potential anti-adhesive activity. It was shown that LHI had a higher relative quantity of monoterpenes than LWE, but LWE showed di-butylphthalide derivatives, which had not been detected in LHI. Di-butylphthalide compounds, which has not been described for *L. officinale* up until now, was detected by LC-MS and are needed to be elucidated further. In addition, both extracts showed a significant reduction (up to 34 %) in bacterial adhesion of UPEC NU14 to T24 bladder cells.

Biomedical studies are powerful methods for identifying pharmacologically active compounds. However, high-throughput screenings in humans and animals are not ethically justifiable. Therefore, a further aim of this study was to develop an *in vitro* cellular screening system using primary murine kidney cells to identify potential THP modulators and to elucidate the biochemical process of THP regulation. Several distal tubule cell isolation protocols were used, while magnetic assisted cell sorting (MACS) seemed to be the most promising method. However, all of these cell-biological studies were unsuccessful, as the primary kidney cells did not survive more than one passage. Controlled and long-term *in vitro* cultivation was not possible by the methods employed and differentiation of the cells to typical Henle cells was not observed.