Renoprotective and Blood Pressure Lowering Impact of *Equisetum arvense* and *Viscum album* Therapy in Experimental Model of Chronic Kidney Disease

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**Abstract**

**Background:** Chronic kidney disease (CKD) is an irreversible decline in the glomerular filtration due to nephrosclerosis and glomerular loss. Rat remnant kidney model enables to assess the benefits of different treatment possibilities.

**Aim:** The aim of our study was to investigate renal morphology and functioning after 12 weeks of treatment with *Equisetum arvense* and *Viscum album*.

**Methods:** Male Wistar rats with 5/6 nephrectomy received herbal drug preparation *Equisetum/Viscum* in a dosage of 0.007 g/kg/die (herbal group) or losartan (180 mg/l, ARB group) or remained untreated.

**Results:** SBP was significantly lower both in herbal group and ARB group compared with untreated animals (p < 0.05). The difference in proteinuria between herbal group and untreated group was significant (p < 0.05), between ARB group and untreated NPX (p = 0.001). Systolic blood pressure (SBP) and proteinuria were measured. Renal cortex tissue samples examined for focal-segmental glomerulosclerosis (FSGS) and interstitial fibrosis (IF). mRNA was isolated to estimate CCL2/MCP-1 gene transcription.

**Conclusion:** We report about beneficial impact of horsetail *Equisetum arvense* and mistletoe *Viscum album* treatment on kidney functioning and morphology.
1. Introduction

Chronic kidney disease (CKD) is an irreversible decline in the glomerular filtration, due to glomerular loss and nephrosclerosis, irrespective of the underlying cause, progress relentlessly. The experimental model of progressive kidney disease (5/6 nephrectomy—NPX) is likewise characterized by severe renal lesions—glomerular hypertrophy, mesangial expansion, focal and segmental glomerular sclerosis, interstitial fibrosis, tubular atrophy and inflammation. NPX model enables the preclinical evaluation of the safety and possible benefits of different strategies, before introducing them in the care of chronic renal patients.

Herbal properties and therapeutic output of horsetail (*Equisetum arvense*) and mistletoe (*Viscum album*) are studied before they have shown diuretic, anti-hypertensive, anti-inflammatory [1] [2] [3], anti-diabetic, anti-toxic and immunomodulating properties. Extracts of *Viscum album* are widely studied for their complementary use in integrative therapy of cancer and other malignant disorders [4]-[18].

Herbal mixture preparation of horsetail *Equisetum arvense* and mistletoe *Viscum album* could be suggested as a complementary advice for chronic kidney disease (CKD) patients (http://www.wala.de/).

The aim of the study was to assess the effect of herbal drug combination *Equisetum* and *Viscum* treatment and to compare it with ARB treatment and non-treatment NPX group.

2. Materials and Methods

2.1. Animals

Male Wistar rats were purchased from the Laboratory Animal Center University of Kuopio, Finland. An acclimatization period of 10 days was allowed before any experiment work was undertaken. Rats were kept in a climate-controlled facility at the Faculty of Medicine of the University of Tartu where animals housed under standard conditions on a 12-h light/dark cycle and fed with standard rodent chow (R 70, Lactamin AB, Sweden) and had free access to tap water. The Animal Studies Ethics Committee in Estonia approved the study protocol.

2.2. Experimental Design

Rats (n = 50), matched for age and body weight were randomly divided into four groups with 10 animals in each. Three groups (n = 30) were subjected to subtotal (5/6) nephrectomy (NPX) as described previously [19]. Randomly selected NPX groups received either ARB (ARB) or herbal treatment (Herbal); one NPX group remained untreated (untreated NPX). One group (n = 10) was sham operated.
control. One group (n = 10) remained un-operated and untreated (Healthy). The animals were monitored for 12 weeks. Systolic blood pressure (SBP, mmHg) was measured biweekly by the tail-cuff manometer (Harvard Apparatus, USA) in conscious pre-warmed rats. Measurement of SBP was repeated six to ten times and average value was noted for each animal. Proteinuria (U-prot, g/24 h) was measured at week 6 and 12 from urine collected for 24 hours using metabolic cages. Blood was collected from the aorta at the end of the study for serum creatinine (S-Crea, μmol/l). The kidney tissue samples were collected at the end of the study for histological examination and part of tissue were snap-frozen in liquid nitrogen for the assessment of MCP-1.

2.3. ARB Treatment

Treatment with ARB (losartan, 180 mg/l) was added to the drinking water and started immediately after the operation. Drug solutions were freshly prepared daily prior to use.

2.4. Herbal Treatment

The animals received orally with drinking water the herbal drug preparation in a dosage of 0.007 g/kg/die using Equisetum/Viscum consisting equal doses of horsetail *Equisetum arvense* and mistletoe *Viscum album* substances that are prepared without alcohol and processed by heating, rhythmising and potentiising by WALA (Arzneimittelverzeichnis). Drug water-solutions were prepared daily prior to use. Treatment started immediately after the operation. Drug was not donated but bought from online pharmacy (eu-versandapotheke.com).

2.5. Morphological Studies

At the end of the study, rats were anesthetized and remnant kidneys removed, fixed in 10% buffered formaldehyde. Paraffin sections of coronal slices, through the pelvis of the remnant kidney, were cut at 4 mm thickness and stained using the periodic acid-Schiff (PAS) and Masson’s trichrome methods.

Kidney sections were studied morphologically for evidence of focal-segmental glomerulosclerosis (FSGS), defined as glomeruli showing evidence of segmental or global collapse of capillaries with or without associated hyaline deposition and adhesions of the capillary tuft to Bowman’s capsule. The extent of FSGS was expressed as a percentage of the total number of glomeruli counted (>50/section).

The presence of interstitial fibrosis (IF) was measured in trichrome stained sections from each kidney and was graded according to the following scale: 0 - no evidence of interstitial fibrosis; 1. <25% involvement; 2. 25% - 50%; 3. >50%.

Quantification of rat MCP-1 mRNA by real time quantitative reverse transcriptase-polymerase chain reaction (RT-PCR):

For quantification of rat MCP-1, or alternatively *Ccl2* (monocyte chemoattractant protein-1 or C-C chemokine ligand 2 in rat) and endogenous reference *β-actin* mRNAs, we used a SYBR Green real-time quantitative RT-PCR method with the ABI Prism 7000 Sequence Detection System (PE Applied Biosystems,....
Foster City, California). Total RNA was extracted from kidney tissue samples by using RNeasy Mini Kit (Qiagen, Hilden, Germany) and cDNA synthesized by a First-Strand cDNA Synthesis Kit SuperScript™ III (Invitrogen). RNA was quantified by determination of ultraviolet absorbance at 260 nm, and purity was assessed by measuring the optical density ratio at 260 and 280 nm (Nanodrop 2000, Thermo Fisher Scientific). Transcript levels for rat MCP-1 (Ccl2) and β-actin were quantified using SYBR Green-based quantitative real-time PCR technology. Amplification was performed by using the RT SYBR Green/ROX qPCR Master Mix (SABiosciences) and Real-Time RT² qPCR Primer Assays. The polymerase chain reaction performed as described by producers [12] [20]. The RT² qPCR oligonucleotide primer sets from SABiosciences are bioinformatically validated and give gene specific PCR amplicon products of the correct size and high amplification efficiency as a result. For amplification quality control we performed the dissociation curve program immediately after the above PCR program and carried out the agarose gel electrophoresis. Amplification efficiencies of endogenous reference and target sequence were comparable. mRNA level of the target sequence were normalized to those of β-actin as a housekeeping gene, and used as an endogenous internal control; the relative levels of each mRNA to that of β-actin were calculated. PCR reactions for both factors were repeated in triplicate.

2.6. Statistical Analysis
Data were analyzed at the end of the study period. Analysis of variance was used in statistical evaluation of the data using SPSS version 14.0. Data is presented as mean ± SD. The relative amount of MCP-1 (Ccl2) transcription in the rat kidney cortex probes was calculated using Delta-Delta Ct method. The difference in transcription level is given by $2^{-\Delta\Delta Ct}$. Differences between groups were examined for statistical significance using Student t-test and Mann-Whitney U test. P < 0.05 was considered significant.

3. Results
Table 1 and Figure 1 present the body weight (BW) of rats measured currently during the study. Increase in weight was smaller in untreated NPX group and the animals significantly smaller at the end of the study (p < 0.05) in untreated group compared with Herbal or ARB group. From NPX group 3 animals and from Herbal group 1 animal died before the end of the study, so these data was excluded from the results. Figure 1 illustrates increase of BW of study groups in timeline.

Average levels of systolic blood pressure (SBP) measured currently during the study showed that in the ARB group and herbal treatment group SBP was significantly lower compared with untreated animals (p < 0.05) as demonstrated in Table 2 and Figure 2.

Serum creatinine elevation was noted in all NPX groups compared with healthy controls and sham operated groups, the elevation was most prominent in the NPX group, although not significantly different from other NPX groups.
Table 1. Body weights (BW, g). Treatment was started at week 0 after surgical NPX. There were no significant differences in BW among the groups at week 0 by ANOVA for repeated measures. At week 6 and week 12 the difference between untreated NPX group and other groups was significant (*p < 0.05). Sham operated and Healthy animals serve as illustrative controls.

<table>
<thead>
<tr>
<th></th>
<th>NPX-untreated</th>
<th>NPX-Herbal treated</th>
<th>NPX-ARB treated</th>
<th>Sham-operated</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>405.5 ± 14.5</td>
<td>349.2 ± 4.8</td>
<td>360.3 ± 3.9</td>
<td>371.4 ± 4.5</td>
<td>360.0 ± 2.8</td>
</tr>
<tr>
<td>Week 2</td>
<td>425.5 ± 4.6</td>
<td>444.5 ± 10.0</td>
<td>388.0 ± 5.0</td>
<td>400.1 ± 3.2</td>
<td>400.0 ± 10.1</td>
</tr>
<tr>
<td>Week 4</td>
<td>430.0 ± 13.8</td>
<td>420.0 ± 12.8</td>
<td>429.5 ± 17.0</td>
<td>458.0 ± 11.7</td>
<td>442.4 ± 14.5</td>
</tr>
<tr>
<td>Week 6</td>
<td>441.1 ± 10.8*</td>
<td>490.9 ± 9.8</td>
<td>482.8 ± 11.2</td>
<td>500.0 ± 12.2</td>
<td>534.0 ± 20.8</td>
</tr>
<tr>
<td>Week 12</td>
<td>466.0 ± 6.7*</td>
<td>567.8 ± 12.3</td>
<td>540.5 ± 19.6</td>
<td>585.8 ± 6.8</td>
<td>576.8 ± 20.8</td>
</tr>
</tbody>
</table>

Table 2. Systolic blood pressure (SBP, mmHg), from week 2 on the difference between untreated NPX group and other groups was significant (*p < 0.05). Sham operated and Healthy animals serve as illustrative controls.

<table>
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<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>128.3 ± 6.2</td>
<td>112.9 ± 9.8</td>
<td>100.0 ± 1.0</td>
<td>98.0 ± 2.0</td>
<td>90.3 ± 12.2</td>
</tr>
<tr>
<td>Week 2</td>
<td>143.0 ± 4.3*</td>
<td>100.0 ± 10.2</td>
<td>90.0 ± 2.7</td>
<td>100.2 ± 9.8</td>
<td>101.5 ± 4.7</td>
</tr>
<tr>
<td>Week 4</td>
<td>150.3 ± 3.8*</td>
<td>96.3 ± 10.3</td>
<td>110.1 ± 6.0</td>
<td>125.2 ± 7.0</td>
<td>113.1 ± 2.7</td>
</tr>
<tr>
<td>Week 6</td>
<td>168.3 ± 8.4*</td>
<td>101.6 ± 8.4</td>
<td>100.2 ± 8.5</td>
<td>112 ± 8.3</td>
<td>98.1 ± 2.0</td>
</tr>
<tr>
<td>Week 12</td>
<td>177.7 ± 4.9*</td>
<td>116.3 ± 18.2</td>
<td>102.3 ± 13.1</td>
<td>104.8 ± 8.4</td>
<td>100.8 ± 8.2</td>
</tr>
</tbody>
</table>

Table 3. Proteinuria (U-Prot, g/24h) and serum creatinine (S-Crea, μmol/l) after 12 weeks of study period. The difference in proteinuria after 12-week period between ARB-treated and untreated NPX rats was significant (*p = 0.001) and between Herbal treated and untreated NPX rats was significant (**p < 0.05) as well. In all NPX groups an increase in serum creatinine was noted in comparison with healthy controls, in the untreated NPX group the increase was most noticeable.

<table>
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<tr>
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<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>U-Prot (g/24h)</td>
<td>10.09 ± 3.13*</td>
<td>5.78 ± 1.40**</td>
<td>2.08 ± 1.01</td>
<td>0.87 ± 0.46</td>
<td>00.00</td>
</tr>
<tr>
<td>S-Crea (μmol/l)</td>
<td>99.40 ± 28.89</td>
<td>60.63 ± 3.45</td>
<td>57.40 ± 5.66</td>
<td>43.20 ± 7.90</td>
<td>31.4 ± 7.23</td>
</tr>
</tbody>
</table>

(Table 3 and Figure 3). Urinary albumin excretion (U-Prot) was also increased in all NPX groups, but was significantly reduced in the ARB and Herbal treatment groups, compared with untreated animals (p < 0.05), data shown in Table 3 and Figure 4.

FSGS and IF estimation in kidney tissue samples of NPX rats revealed that significantly less glomerulosclerosis was found and IF score was lower both in ARB-group and herbal group compared to untreated group as shown in Table 4, Figure 5 and Figure 6.
Table 4. Focal-segmental glomerulosclerosis (FSGS), interstitial fibrosis (IF) and MCP-1 gene relative transcription in kidney tissue of NPX rats after 12 weeks of study period. The difference in FSGS between ARB-treated and untreated NPX rats was significant (*p = 0.001) and between Herbal treated and untreated NPX rats was significant (*p = 0.001) as well. The difference in MCP-1 relative transcription after 12-week period between Herbal treated and untreated NPX rats was significant (*p = 0.001). Between ARB-treated and untreated NPX rats as well as sham-operated and healthy control was significant (**p < 0.05) as well.

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>FSGS (%)</td>
<td>31.17 ± 2.87</td>
<td>7.11 ± 1.29*</td>
<td>5.12 ± 2.3*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IF (0…3)</td>
<td>1.33 ± 0.48</td>
<td>1.02 ± 0.11</td>
<td>0.1 ± 0.1*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCP-1 relative transcription</td>
<td>3417 ± 2240</td>
<td>1680 ± 862*</td>
<td>1038 ± 588**</td>
<td>974 ± 236**</td>
<td>1344 ± 577**</td>
</tr>
</tbody>
</table>

Figure 1. Gain in body weight during the study period of 12 weeks. The weight of NPX animals was significantly lower after 12 weeks.

Figure 2. Changes in systolic blood pressure during the study period of 12 weeks. The SPB of untreated rats was significantly higher from week 4 in comparison with other groups.

Quantitative RT-PCR analysis revealed that the differences of mRNA transcription for MCP-1 between ARB treatment and untreated group were
Figure 3. Serum creatinine at the end of the study. Serum creatinine elevation was noted in all NPX groups compared with healthy controls and sham operated groups, the elevation was most prominent in the NPX group, although not significantly different from other NPX groups.

Figure 4. Urinary albumin excretion (U-Prot) was also increased in all NPX groups, but was significantly reduced in the ARB and Herbal treatment groups, compared with untreated animals (p < 0.05).

Figure 5. FSGS at the end of the study. Significantly less glomerulosclerosis was found score was both in ARB-group and herbal group compared to untreated group.
statistically highly significant ($p < 0.001$), gene transcription was more than three times higher in untreated animals. Differences were revealed between herbal therapy and untreated group, being higher in untreated animals ($p < 0.05$). Transcription differences according to the qPCR data between study groups are presented in Table 4 and Figure 7.

4. Discussion

We aimed in a 12-week experimental study in rats with 5/6 renal mass ablation (NPX) to compare the effect of ARB treatment and herbal mixture treatment of horsetail (*Equisetum*) and mistletoe (*Viscum*) in CKD experimental model with IF at the end of the study. Significantly less interstitial fibrosis was found in ARB group compared to untreated group.

**Figure 6.** IF at the end of the study. Significantly less interstitial fibrosis was found in ARB group compared to untreated group.

**Figure 7.** Quantitative RT-PCR analysis revealed that the differences of mRNA transcription for *MCP-1* between ARB treatment and untreated group were statistically highly significant ($p < 0.001$), gene transcription was more than three times higher in untreated animals. Differences were revealed between herbal therapy and untreated group, being higher in untreated animals ($p < 0.05$).
early development of hypertension and proteinuria and with progressive glomerulosclerosis, interstitial fibrosis, tubular atrophy and inflammation in remaining kidney tissue. Various large-scale studies assessing ARB treatment indicate that ARB slows the pace of progressive renal injury resulting from CKD of diverse aetiologies [19] [21]-[27].

Our study assessed and compared the impact of herbal treatment with horsetail (Equisetum) and mistletoe (Viscum) in NPX rats. Herbal properties and therapeutic output of both herbs are studied previously [4] [9], but there are no previous experimental studies about this particular combination of herbs, although, Masteikova R et al. suggest in an experimental study that the effect of herbal combination therapy with horsetail is better than the single herb alone [28].

We measured currently SPB and renal functional parameters, studied renal patomorphology and CCL2/MCP-1 gene transcription in renal tissue in NPX rats treated 12 weeks with herbal drug preparation in a dosage of 0.007 g/kg/die using Equisetum/Viscum consisting equal doses of horsetail Equisetum arvense and mistletoe Viscum album substances that are prepared without alcohol and processed by heating, rhythmisising and potentising by WALA (http://www.walaremedies.de/).

We compared the results with matched NPX controls remained untreated and with healthy animals.

We observed significantly lower blood pressure level in herbal therapy group in comparison with untreated group. We saw the tendency to lowering of proteinuria in herbal therapy group as well. Thus, we could speculate about ameliorating of glomerular hemodynamics due to herbal treatment.

Herbal treatment with horsetail and mistletoe, as described in methods, had modulated MCP-1 gene transcription.

We observed significant reduction of MCP-1 mRNA transcription compared with untreated animals, almost to the level of healthy control group. In addition to significant reduction of MCP-1, could be partly due to the impact on the pro-inflammatory gene transcription [29].

We are aware of several limitations concerning herbal treatment investigations. There are various techniques of how herbal drugs are prepared for administration. It is difficult to compare different studies. The quality of preparation of herbal drug is of outmost importance. The reason we used the combination medicine of WALA Remedies, Germany, is because of their ultimate care of quality and long practice in herbal drugs (http://www.walaremedies.de/).

Phytopharmaceuticals are common adjuvant therapies, but as complex mixtures with multiple targets they have not been systematically investigated. There is still need to have more evidence to provide scientific credence for the use of herbal medicines. It is important to investigate various beneficial mechanisms that could protect kidney tissue from progressive damage and could contribute to decelerate the progression of CKD. Our study with remnant kidney model of
progressive kidney disease shows possible benefits of herbal treatment with mixed herbal solution of horsetail and mistletoe.

5. Conclusion

Based on the results of our study, we conclude that: SBP was significantly lowered; urinary albumin excretion was significantly reduced; estimated FSGS was significantly less prominent and MCP-1 gene transcription in renal tissue was lower in studied rat group treated with herbal combination of horsetail and mistletoe than in untreated NPX group.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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