

# Effect of Herbal Medicine *Achillea Millefolium* on Plasma Nitrite and Nitrate Levels in Patients With Chronic Kidney Disease A Preliminary Study

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**Keywords.** *Achillea*, chronic kidney disease, nitric oxide, hemorrhage

**Introduction.** Increased plasma nitric oxide concentration has been supposed as one of the possible mechanisms of bleeding tendency in patients who suffer chronic kidney disease. Nitric oxide-scavenging properties have been reported with some *Achillea* species. This study was designed to find any possible effect of *Achillea millefolium* on plasma nitric oxide concentration in these patients.

**Materials and Methods.** Thirty-one chronic kidney disease patients were included in this randomized controlled trial, of whom 16 received 1.5 g of powdered *A millefolium* flower 3 days a week for 2 months, and 15 received placebo for the same period. Plasma samples were collected before and after the study period to estimate the effect of *A millefolium* on plasma nitric oxide metabolites (nitrite and nitrate).

**Results.** Although not statistically significant, plasma nitrite and nitrate concentrations decreased after 2 months' administration of *A millefolium* ( $0.82 \pm 0.51 \mu\text{mol/L}$  to  $0.63 \pm 0.42 \mu\text{mol/L}$  and  $50.55 \pm 17.92 \mu\text{mol/L}$  to  $44.09 \pm 17.49 \mu\text{mol/L}$ , respectively). These concentrations were slightly increased in the placebo group after the study period.

**Conclusions.** Countercurrent to the placebo group, plasma nitric oxide metabolites were marginally decreased after *A millefolium* administration in chronic kidney disease patients. Higher doses or longer duration of plant administration may make these changes more significant.

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## INTRODUCTION

Amongst many complications associated with chronic kidney disease (CKD), uremic bleeding is a well-known syndrome caused by multiple factors, including platelet dysfunction. While there are reports of nitric oxide (NO) deficiency in CKD patients,<sup>1</sup> some strongly suggest that the plasma concentration of NO is higher in uremic patients compared to nonuremic ones.<sup>2</sup> Some investigations propose that excessive NO production may inhibit platelet aggregation.<sup>2, 3</sup>

Numerous treatment methods have been suggested to manage uremic bleeding. Among them administration of conjugated estrogens is proved to be a safe and effective method. Although the exact mechanism by which estrogen corrects bleeding tendency in uremia is still unclear, it has recently been suggested that 17 $\beta$ -estradiol (the most active component) reduces the concentration of NO by decreasing its production.<sup>4,5</sup>

Use of medicinal plants dates back to ancient times. Herbal medicine provides plentiful

alternatives to synthetic compounds in the treatment of almost any condition, as it is believed to be less toxic. *Achillea millefolium* L, (*Asteraceae*), commonly known as yarrow, is an ancient remedial herb used to treat wounds, hemorrhages, spasmodic diseases, inflammation, and pain.<sup>6</sup> *A millefolium* is widely distributed and native to various regions of Iran. Although some references suggested antihemorrhagic properties for *A millefolium*, there is yet no precise pharmacological mechanism explaining this effect.<sup>7</sup> Investigations on extracts of other *Achillea* species like *A santolina* have shown antioxidant and free radical scavenging potential including decrease in serum NO concentration.<sup>8,9</sup> The total amount of NO in plasma can be assessed using the stable oxidation metabolites of NO, nitrite and nitrate, with dietary NO control.<sup>1</sup> Regarding the role of NO in bleeding process of CKD patients, the present study was designed to assess the possible effect of this medicinal herb on plasma nitrite and nitrate levels as indicators of NO concentration in CKD patients.

## MAERIALS AND METHODS

### Herb and Placebo Preparation

Aerial parts of the plant, including the flowers, were gathered in May and were characterized as *A millefolium* by the Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences, Iran. These parts were cleaned from dirt, sand, bugs, and excess leaves with delicacy, and flowers were grinded finely. Size-0 gelatin capsules were filled with powdered flowers manually and were measured to weigh 500 mg. Pharmaceutical-grade lactose (Merck, Darmstadt, Germany) was used to fill placebo capsules with the same size and color.

### Clinical Study

To perform this preliminary randomized, double-blinded, placebo-controlled clinical trial, medical data of more than 60 patients with CKD were reviewed in the nephrology clinic of Imam Khomeini Hospital Complex, affiliated to Tehran University of Medical Sciences. Considering the least of differences between NO concentrations before and after herb or placebo administration as 1 mark, the reported standard deviation of 0.9  $\mu\text{mol/L}$ ,<sup>9</sup> the confidence level of 0.05, and the power as 90%, the sample size was calculated

to be 10 patients in each group according to the following formula.

Patients with CKD and a glomerular filtration rate between 15 mL/min/1.73 m<sup>2</sup> and 60 mL/min/1.73 m<sup>2</sup> with no active bleeding disorders were included in this study. Receiving medicines known to change nitrate and nitrite plasma levels (including nitrate agents, phosphodiesterase inhibitors, conjugated estrogen, and *Ginko biloba*), anticoagulant medications, and antiplatelet medications were considered as exclusion criteria, as well as hypersensitivity to yarrow or lactose, pregnancy, and lactation. The study protocol was approved by the local ethics committee of Tehran University of Medical Sciences and all patients provided informed consent.

Patients were divided into 2 groups using permuted block randomization. Patients in group 1 received *A millefolium* and those in group 2 received lactose as placebo. To control dietary source of nitrite and nitrate, all participants were asked not to make significant changes in their diet. Group 1 received 3 capsules (1.5 g) of yarrow per day, 3 times a week for 2 months. The other group received the same amount of lactose as placebo. Venous blood samples were collected in heparinized tubes at the initiation of the study and subsequently after two months of the study period. Blood samples were centrifuged at 2500 rpm for 10 minutes immediately after collection. Plasma tubes were then frozen at -20°C until analysis for approximately 6 months. All patients were monitored regarding adverse herb reaction including hypoglycemia and allergic reactions.<sup>7</sup>

### Determination of Plasma Nitrite and Nitrate

Plasma levels of nitrite and nitrate were measured using a newly developed precolumn high-performance liquid chromatography method. In this method, nitrite was derivatized with Griess reagents, sulfanilamide, and *N*-(1-naphthyl) ethylenediamine. Later, the molar absorptivity for the azo dye formed in the diazotization reaction was measured at 540 nm. Lower limit of quantification for this Griess-high-performance liquid chromatography method was 66.7 nM of nitrite.<sup>10</sup>

### Statistical Analyses

Data was analyzed using the SPSS software (Statistical Package for the Social Sciences, version

11.0, SPSS Inc, Chicago, Ill, USA). Nonparametric tests were used for analysis. The Mann-Whitney test was used to compare nitrite and nitrate concentrations at the initiation and at the end of the study between the two groups. The Wilcoxon test was used to compare plasma nitrite and nitrate levels of CKD patients at the initiation of the study with those at the end of two months trial within each group. Statistical significance was considered as a *P* value less than .05. All continuous data are presented as mean ± standard deviation.

**RESULTS**

A total of 31 patients (16 in group 1 and 15 in group 2) were included in this study. One patient in group 1 was excluded from the study due to *A millefolium*-induced skin rash. Therefore, 15 patients in each group completed the experiment. Table 1 shows the demographic characteristics of patients in both groups. As seen, there was no difference between the two groups regarding age, weight, mean estimated glomerular filtration rate, and

history of diabetes mellitus or hypertension.

Plasma nitrite and nitrate concentrations at the initiation and the end of the study have been presented in Table 2. The mean plasma concentrations of basal nitrite and nitrate decreased during the two-month administration of *A millefolium* and conversely increased in the placebo group; however, these differences did not reach statistical significance.

**DISCUSSION**

Chronic kidney disease is associated with many complications, including increased bleeding tendency possibly due to platelet dysfunction. Abnormalities in platelets’ performance may be attributable to elevated production of prostacycline and nitric oxide.<sup>4,11</sup>

*Achillea millefolium* was named after *Achilles* who was the first to use this plant as a remedy to stop the bleeding from his warriors’ injuries.<sup>12,13</sup> Yarrow is native to vast regions of the world and its production for commercial use has been experimented before,<sup>14</sup> so it seems to be not a costly product to be applied in practice. In addition, yarrow can be used in various dosage forms which results in better patient compliance. Although extracts and tinctures of *A millefolium* are more concentrated than the dried powdered plant, their bitterness and pungent odor may repel the consumer. Therefore in this research, yarrow flowers were filled in capsules to mask their odor and taste. The effective dose of 2 g to 4 g of *A millefolium* dried flowers per day in a healthy normal person is suggested in a herbal medicine reference.<sup>15</sup> Since the hepatic or renal elimination of *A millefolium* from the body has not been established yet, the dose of 1.5 g per day was chosen to be experimented on patients with CKD. Also, to investigate plant’s prolonged effect, the administration of herbal capsules continued for 2 months.

*A millefolium* has kept its promising effects throughout history and is still being used for its antibleeding properties,<sup>7</sup> but the exact mechanism of this effect has not been revealed yet. Other species of *Achillea* have shown anti-oxidant and free radical scavenging properties.<sup>16</sup> Some of them were shown to reduce the level of nitric oxide in uremic rats.<sup>8</sup> According to Noris and coworkers, prolonged bleeding can be corrected

**Table 1.** Characteristics of Patients on *Achillea millefolium* and Placebo\*

Characteristic	<i>Achillea millefolium</i>	Placebo	<i>P</i>
Gender			
Male	9	11	
Female	6	4	.69
Mean age, y	50.20 ± 17.06	52.40 ± 9.40	.34
Mean weight, kg	71.10 ± 10.35	70.30 ± 10.54	.92
Mean serum creatinine, mg/dL	2.82 ± 0.92	2.66 ± 0.98	.21
Mean GFR, mL/min/1.73 m <sup>2</sup>	23.70 ± 6.62	30.75 ± 10.68	.09
Diabetes, %	6	5	> .99
Hypertension, %	5	5	> .99

\*GFR indicates glomerular filtration rate.

**Table 2.** Nitrite and Nitrate Concentrations at the Initiation and End of Study

Parameter	<i>Achillea millefolium</i>	Placebo	<i>P</i>
Nitrite, µmol/L*			
Baseline	0.82 ± 0.51	0.62 ± 0.38	.53
Two months	0.63 ± 0.42	0.83 ± 0.42	.47
Nitrate, µmol/L†			
Baseline	50.55 ± 17.92	37.87 ± 29.08	.28
Two months	44.09 ± 17.49	45.18 ± 22.79	.71

\*Before-after comparisons: *P* = .13 for the *Achillea millefolium* group and *P* = .86 for the placebo group.

†Before-after comparisons: *P* = .44 for the *Achillea millefolium* group and *P* = .99 for the placebo group.

by conjugated estrogens.<sup>5</sup> On the other hand, Innocenti and colleagues investigated in vitro estrogenic activity of *A millefolium* and suggested two compounds with slight estrogenic activity.<sup>17</sup> Although the present study cannot explain the effects of *A millefolium* by correlating it to the well-known effect of estrogens, this could be a hint for further inquiries. Even though this study failed to demonstrate statistically significant results, the findings interestingly showed that levels of nitrite and nitrate decreased in CKD patients who received *A millefolium* while the concentrations of these two ions increased in patients receiving placebo. Decrease in nitrite and nitrate contents of plasma is in harmony with findings of a previous study carried out by Yazdanparast and associates.<sup>9</sup>

Small sample size, low doses of *A millefolium* and short duration of administration are limitations that need to be addressed regarding the present study. Despite the fact that ordering higher doses of *A millefolium* or decreasing the intervals may yield to statistically significant results, it cannot be done abruptly, considering the inadequate data on pharmacokinetic and pharmacodynamic properties of *A millefolium* in humans. Hence, we estimated a nontoxic dose to be used in patients with impaired kidney function. Instead of using high doses of yarrow, smaller amounts were administered in an extended duration. During the experiment, all participants were monitored for *A millefolium*'s adverse reactions or overdose symptoms. While there were no cases of overdose symptoms, adverse reaction of skin rashes was observed in one female subject who was excluded from the study.

## CONCLUSIONS

This is a preliminary study with findings that although insignificant, it showed that *A millefolium* administration resulted in decrease in plasma nitrite and nitrate concentrations whereas plasma nitrite and nitrate levels increased in the placebo group. These data suggest nitric oxide scavenging properties of this herb in CKD patients. The main limitations of this study are small sample size and lack of bleeding time monitoring. The latter was due to the fact that subjects in this study were outpatients that referred to different laboratories that confounded subjective test of bleeding time monitoring. We propose supplementary studies

including larger sample size of CKD patients tend to bleed, administration of higher doses of *A millefolium*, and monitoring bleeding time uniformly in these patients in order to justify the anti-hemorrhage property of this ancient medicinal plant in patients suffering chronic kidney disease. The reported treatment dose, duration, and safety in this study are guides for designing treatment regimen in future studies.

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## CONFLICT OF INTEREST

None declared.

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