The Electrochemical Study of the Effects of Herbal Tea on Calcium Oxalate Renal Stones

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**INTRODUCTION**

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Urinary stone formation affects 10-12% of the population in industrialized countries. Their prevalence has increased in recent years, while the age of onset has decreased. Diet and environment play an important role in kidney stone diseases, possibly by changing the composition of the urine [1-3].

Renal stones have different types, including calcium oxalate, calcium phosphate, ureic acid, magnesium ammonium phosphate (Struvite) and cysteine stones, that about 75-80% of urinary stones are composed of calcium oxalate and calcium phosphate salts [4-10]. Of course, other unusual kidney stones such as xanthine, dihydroxy adenine, silicate and matrix have also been reported [10-16].

However, for the treatment of patients with kidney stones, various therapies such as the use of chemical drugs or surgeries have been used, but, so far, there is no effective and safe drug treatment that results in complete treatment or prevention of stone formation [17-23]. Today, because of the side effects and harmful effects of chemical drugs, the use of herbal products has attracted the attention of researchers [24-31].

In recent researches, the effects of some medicinal herbs such as barberry, Nigella Sativa, Juniperus excelsa and Allium jesdanum have been investigated in clinical or laboratory form on kidney stone. This study was conducted to investigate the effect of Cleome dolichostyla extract on the formation and dissolution of calcium oxalate kidney ston. Cleome dolichostyla is an annual herb, which grows wild in barren lands and belongs to the family Cleomaceae. This plant contains some mineral compounds, including Ca, Mg, Fe, Zn, Cu and Mn and fatty acids such as linoleic acid, palmitic acid, stearic acid and linolenic acid [31-37].

In this research, at first, Cleome dolichostyla plant was collected from the county of Lahore province, and then washed some of plant with double distilled water and dried in laboratory temperature and away from sunlight for 4 days, then weighed the amount of 5.0 g of leaf and seed of the plant and mixed with 75.0 ml of double distilled water at 65 ° C temperature and placed them toward the steam of boiling water for 120 minutes. The resulting blends were filtered with Whatman filter paper, and extended the solution volume under the filter to 100 ml with the help of double distilled water. At the end, we put the resulting aqueous extracts at a temperature of 4.0 ° C for conducting relevant experiments. The materials used in the titration are: Sodium oxalate (Na₂C₂O₄), Dihydrate calcium chloride (CaCl₂.2H₂O), Ammonia) NH₃, Hydrochloric acid (HCl), Ethylenediaminetetraacetic acid (EDTA), Calcium oxalate (CaC₂O₄), Eriochrome Black T, Double-distilled water [21-23].

Meanwhile, ammonia buffer solution with pH =10.0 was prepared from adding the hydrochloric acid to ammonia solution and adjusting by pH meter. Standard calcium solutions with specific densities were prepared by dissolving the appropriate amounts of calcium chloride in double distilled water (Fig. 1).

RESULTS AND DISCUSSION

The result of the end points (Equivalence) obtained from the complexometry titration of the solutions that were mentioned above with the EDTA standard solution are presented in Table 1 and 2. The consumption volume of EDTA for the solution of the aqueous extract of Cleome dolichostyla leaves and seeds, saturated with calcium oxalate, in comparison with their total volume of consumption in the titration of the two blank and extract solutions lacking calcium oxalate shows a significant increase (p<0.05).

MATERIAL AND METHODS
Fig. 1. The process of extracting and complexometric titration of the Cleome dolichostyla

Fig. 2. The process of complexometric titration of the blank

Table 1. Results of complexometric titration of Cleome dolichostyla leaves with EDTA(0.005M)

<table>
<thead>
<tr>
<th>Titrand</th>
<th>Blank</th>
<th>Extract without CaC₂O₄</th>
<th>Extract with CaC₂O₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of titrant (EDTA)</td>
<td>0.3</td>
<td>9.3</td>
<td>10.1</td>
</tr>
</tbody>
</table>

Table 2. Results of complexometric titration of Cleome dolichostyla seeds with EDTA(0.005M)

<table>
<thead>
<tr>
<th>Titrand</th>
<th>Blank</th>
<th>Extract without CaC₂O₄</th>
<th>Extract with CaC₂O₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of titrant (EDTA)</td>
<td>0.3</td>
<td>8.5</td>
<td>10.0</td>
</tr>
</tbody>
</table>
Comparison of the volume of consumption of sodium oxalate solution related to the points of equivalence showed a significant difference (p < 0.05).

CONCLUSION

In this research, with the aim of achieving a herbal medicine with the least side effects, we study the effect of aqueous extract of leaf and seed of *Cleome dolichostyla* plant on the formation and dissolution of calcium oxalate kidney stones. This is important because in pharmacy science, a comprehensive chemical medicine which can prevent the formation of kidney stones and treating them with the least effects is not available. Since most kidney stones are generally calcium oxalate, this compound has little solubility in water ($K_{sp} = 1.7 \times 10^{-9}$). Therefore, the presence of calcium ions ($Ca^{2+}$) and oxalate ($C_2O_4^{2-}$), even at relatively low density in the kidney's aquatic environment, can lead to calcium oxalate formation or in other words, kidney stones [27-30].

Meanwhile, if we can use some herbal extracts to incorporate compounds into the
kidney environment which those compounds in the interaction with renal salts prevent the formation or dissolution of the sediment, we have taken an effective step in the treatment and prevention of kidney stone disease. Therefore, in this research, the effect of Cleome dolichostyla on the formation and dissolution of calcium oxalate stones has been studied. In this regard, two methods of complexometry and conductometric titration have been used.

In the complexometry method, the density of Ca$^{2+}$ existed in the solution in the presence of Eriochrome Black T detector was determined by gradual increase of EDTA ligand in an ammonia buffer environment with pH=10 based on the following reaction:

$$\text{Ca}^{2+} + \text{EDTA}^4- \leftrightarrow [\text{Ca}(\text{EDTA})]^2-$$

In the test related to the leaf and seed of the plant, in the presence of the extract, this change is from light brown to slime green. The end point of titration for non-extract blank solutions is obtained by changing the color of the solutions from violet to blue [31-32].

The results of the complexometry presented in Table 1 and 2 show that the volume of EDTA used in the titration of the aqueous extract of leaf and seed of calcium oxalate (10.1 and 10 ml, respectively), which is higher for both extracts than the total volume of EDTA for the blank (0.3 ml) and the non-calcium oxalate extracts (9.3 and 8.5 ml, respectively). Therefore, for calcium density in solutions, the following relation is true:

$$\mu\text{mol} \text{Ca}^{2+}(\text{leaf extract containing CaC}_2\text{O}_4) > [\mu\text{mol} \text{Ca}^{2+}(\text{blank}) + \mu\text{mol} \text{Ca}^{2+}(\text{leaf extract without CaC}_2\text{O}_4)] 10.1 > 0.3 + 9.3$$

$$\mu\text{mol} \text{Ca}^{2+}(\text{seed extract containing CaC}_2\text{O}_4) > [\mu\text{mol} \text{Ca}^{2+}(\text{blank}) + \mu\text{mol} \text{Ca}^{2+}(\text{seed extract without CaC}_2\text{O}_4)] 10.0 > 0.3 + 8.5$$

If the extract in the solution increases the solubility of calcium oxalate salt, we expect that the calcium ion density calculated in titration with EDTA is higher than the blank solution. Possibly, the presence of some interactions between the active ingredients of the extract with oxalate and calcium ions increases the dissolution of calcium oxalate salts. Therefore, it can be concluded that the aqueous extract of leaf and seed of both cases increased the dissolution of calcium oxalate in the solution. Therefore, in patients with calcium oxalate kidney stones, these extracts can be recommended to dissolve kidney stones. Another important issue for kidney stone disease is the problem of recurrence and getting afflicted to kidney stones in people who has improved. Therefore, in this research, we used the conductometric titration method to study the effect of aqueous extract of leaf and seed in the process of preventing the formation of calcium oxalate sedimentation. In this titration, with the gradual increase of the titrant to the titrating solution, the electrical conductivity is measured by the conductivity meter, which has a direct relation with the density and type of ions presented in the solution.

In this research, the effect of the presence of extracts in the process of the formation of calcium oxalate sedimentation in calcium ion solutions was investigated in titration with sodium oxalate solution. Since the effect of titration for each calcium ion of Ca$^{2+}$two Sodium ion Na$^+$ is introduced into solution, therefore the electrical conductivity of the solutions is gradually increased with gradual increase of sodium oxalate solution until the sediment gradually and with gentle slope is completed. After the equivalence point and with the continuation of the titration, the sedimentation process is completed and, therefore, without the removal of ion from the solution, only the density of both sodium and oxalate ions increases in such a way that it compensates for the dilution of the solution due to increased volume, so the electrical conductivity...
conductivity increases with a steeper slope. Fig. 3 and 4 show the changing process of the electrical conductivity of the reading (blank, the extract of leaf and seed *Cleome dolichostyla*) containing calcium chloride as compared to the titrated sodium oxalate. The study of these titrations shows that the consumption volume of titrated sodium oxalate solution for the samples containing both extracts is higher than the blank sample. In the complexometric titration, firstly 5.0 ml of aqueous extract of leaf and seed of *Cleome dolichostyla* was mixed with 20 ml of double distilled water and 0.01 g of solid calcium oxalate. Both mixtures were mixed at room temperature for 90 minutes by magnetic stirrer and were passed through a glass filter. Subsequently, 3.0 ml of ammonia buffer was added to each of the following solutions and titrated in the presence of Eriochrome Black T reagent with EDTA 0/005 M. The volume end points of these titrations were compared with consumption volumes of EDTA in the titration of blank solutions (saturated solution of calcium oxalate in water) and solutions without calcium oxalate [16-19]. In conductometric titration by using JENWEY conductivity meter (Model 4510), first separately, 25.0 ml of calcium chloride solution of 0.025 M containing aqueous extract of leaf and seed was prepared and stirred at room temperature for 90 minutes and filtered with a glass filter. Then conductometric titration of under the filter solution and the blank solution (a solution of 0.025 M calcium chloride in water) with sodium oxalate 0.025 M was performed.

ACKNOWLEDGMENTS

We want to appreciate the department of herbal medicine at the University of Lahore for their great supports.

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