Chapter 5

Prophylactic and curative effect of *Scoparia dulcis* on kidney stone induced male Wistar rats

5.1 INTRODUCTION

In the previous chapter we have discussed about the *in vitro* effect of the drug *Scoparia Dulcis* in inhibiting the growth of struvite urinary crystals. The study proved that the drug is effective in inhibiting the struvite crystals. To have detailed information about the drug’s effect in human body, further studies have been done in male Wistar Rats.

*In vivo* experimental studies are done by inducing kidney stones in the Wistar rats and modulation of disease progression was noted after intervention of extract. Instead of struvite crystals, here the inhibition effect is tested against the growth of calcium oxalate monohydrate crystals. The *in vivo* studies are categorized into prophylactic effect study and curative effect study of the drug. The prophylactic effect (prevention) proves that whether the prior intake of the drug or the prior presence of chemical components of the drug in the human body can stop or reduce the growth of urinary crystals. The curative effect (treatment) proves the intake of this drug by a patient, who is affected by kidney stones, will reduce the kidney stone size and act as a medicine to cure this condition.

In this chapter we are going to discuss about the prophylactic and curative effect of *Scoparia Dulcis* in calcium oxalate induced male Wistar Rats. The study of drug effect on rats has been a main research area in drug designing section. Rather than the drug’s effect on treating the disease, its effect on all the nearby organs will also be studied parallel.

*The work discussed in this chapter has been published

5.2 EXPERIMENTAL PROCEDURE

5.2.1 ANIMALS AND STUDY DESIGN

Twenty four male Wistar rats, weighing approximately 150 - 200 g were used in the present experimental study. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (Registration Number - 1333/c/10/CPCSEA; Ethical Clearance Number - VIT/IAEC/8th/07). The animals were housed in polypropylene cages and maintain under the standard conditions of 12 hours dark/light cycle at 27±1 °C.

The animals were divided into four groups and were kept in four different cages. The normal diet was given to the rats in the first cage, which was the control group, Group I. The drinking water was replaced by the solution of 1% ethylene glycol in water (Lyon E.S et al., 1996) for the Group II. The Group III animals were ingested with ethylene glycol solution and the extract (50mg/kg) was supplemented simultaneously for 22 days, to study the prophylactic effect of the drug. In Group IV, the animals were supplied with ethylene glycol solution for 22 days to induce the stone and the extract was administered (50mg/kg) from 22nd to 42nd day to observe the curative effect.

5.2.2 BIOCHEMICAL ANALYSIS

The twenty-four hour urine samples were collected by keeping the rats in the metabolic cages. The blood was collected from the rats in each group after euthanizing the animals and stored without anticoagulant to get the serum. The biochemical analyses for the urine and serum samples were done to find the quantity of calcium, magnesium, phosphate, creatine and urea using standard kits.

5.2.3 HISTOPATHOLOGY STUDY

At the end of the study period the rats were euthanized by cervical dislocation under anesthesia. Kidney and liver were carefully removed and perfused using phosphate buffer saline (PBS). The cleaned organs were fixed in 10% formalin solution. Section were cut with 4 µm thicknesses using 4 Leica RM 2126 microtome and mounted on
slides after staining with Haematoxylin & Eosin (H & E). The sections were focussed using a microscope of magnification 400x and the photographs were taken.

5.2.4 STATISTICAL ANALYSIS

The results of the serum and urine analysis were expressed in terms of mean ± S.D. The results were done One-way ANOVA test and the results are said to be significant for probability value p < 0.05.

5.3 RESULTS AND DISCUSSION

5.3.1 ANALYSIS OF URINE PARAMETERS

The 24-hr urine has been collected from all the animals of all the groups. The urine parameters were quantitatively analyzed and given in Table 5.1.

Table 5.1: The changes in urine parameters in control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>3.47 ± 0.03</td>
<td>4.09 ± 0.26**</td>
<td>3.765 ± 0.13b*</td>
<td>3.595 ± 0.06**</td>
</tr>
<tr>
<td>Magnesium</td>
<td>5.99 ± 0.80</td>
<td>2.99 ± 0.63**</td>
<td>5.89 ± 0.37**</td>
<td>3.746 ± 0.25**</td>
</tr>
<tr>
<td>Phosphate</td>
<td>3.89 ± 0.69</td>
<td>4.10 ± 0.45**</td>
<td>1.74 ± 0.03**</td>
<td>2.60 ± 0.24**</td>
</tr>
<tr>
<td>Oxalate</td>
<td>4.52 ± 0.18</td>
<td>6.39 ± 0.39**</td>
<td>5.05 ± 0.42**</td>
<td>5.23 ± 0.16**</td>
</tr>
</tbody>
</table>

All the parameters were expressed as mg/dL. The values are expressed as mean ± SD of four animals and results are statistically analysed by one way ANOVA with Bonferroni's multiple comparison post test (n=6). The comparisons are made as follows: ‘a’ – Control Vs Diseased; ‘b’ – Diseased Vs Prevention; ‘c’ – Diseased Vs Treatment. *** p<0.001, ** p<0.01, * p<0.05, NS – Not Significant.

The calcium level has been increased in diseased group II compared to the group I, which occurs due to excessive tubular damage in the kidney, which may lead to excretion of intracellular calcium. However, during the administration of extract the calcium level was managed in Group III and IV, which proves that extract is effective
in inhibiting hypercalciuria. Magnesium reduces super saturation and so, it is considered as one of the potent inhibitors of calcium oxalate crystallization (Rushton and Spector et al., 1982). Magnesium level was significantly decreased in Group II animals compared to normal animals, due to metabolic acidosis and super saturation. When the administration of the extract was started, magnesium level was improved in Group III and IV animals in order to reduce the concentration of calcium. The level of magnesium was in preventive group reached almost normal value while the curative therapy showed a mild improvement than the infected animals. Hypercalciuria leads to increased phosphate leakage and in the diseased rats it was only a slight increase in comparison to control rats (Selvam and Varalakshmi, 1989). In treated rats decreased in level of phosphate was also at par to control rats. Formation of CaOx crystals can be well exhibited from the obvious increase in urinary oxalate level in the ethylene glycol ingested rats compared to control Group I. The effect of the extract can be well demonstrated by its potency in treating hyperoxaluria. This has been proved by the decreased level of oxalate in Group III and IV.

5.3.2 ANALYSIS OF SERUM PARAMETERS

The serum collected from the experimental animals was analyzed and the quantity of various parameters has been calculated and noted in Table 5.2. The value of urea and creatinine is significantly (p < 0.05) increasing in Group II, III and IV compared to the control Group I, this shows that a renal function has been during the calcium oxalate crystallization. The urea and creatinine level has been shown a significant decrease in treating Group III and IV compared to diseased group II, which shows the ability of the extract to offer nephro protection which is mainly due to various antioxidants and anti-inflammatory compounds present in it (Hayashi et al., 1991).

Serum calcium level was mildly decreased in the Group II animals compared to the control rats, corresponds to excessive urinary excretion. This was very well improved in group III and IV animals. The calcium level seems to be increasing in the treated groups compared to group II, which proves the effect of inhibition of calcium crystal formation by the extract.
Table 5.2: The changes in serum parameters in control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>29 ± 0.94</td>
<td>53 ± 0.85**</td>
<td>31.1 ± 1.12***</td>
<td>30.3 ± 0.75***</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.35 ± 0.06</td>
<td>0.65 ± 0.06<em>a</em></td>
<td>0.4 ± 0.07**</td>
<td>0.35 ± 0.04***</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.6 ± 0.24</td>
<td>8.4 ± 0.38**NS</td>
<td>9 ± 0.40**NS</td>
<td>9.4 ± 0.16**NS</td>
</tr>
</tbody>
</table>

All the parameters were expressed as mg/dL. The values are expressed as mean ± SD of four animals and results are statistically analysed by one way ANOVA with Bonferroni’s multiple comparison post test (n=6). The comparisons are made as follows: ‘a’ – Control Vs Diseased; ‘b’ – Diseased Vs Prevention; ‘c’ – Diseased Vs Treatment. *** p<0.001, ** p<0.01, * P<0.05, NS – Not Significant.

5.3.3 HISTOPATHOLOGY STUDIES

The microscopic images of kidney and liver sections of various groups are given in Fig 5.1. According to the histopathology observations, the intake of ethylene glycol has induced polymorphic irregular crystals in the kidneys of Group II animals (Fig 5.1 c) with early tubular necrosis. While the liver section of the diseased animal shown in Fig 5.1 (d), demonstrates sinusoidal and venular dilation. In prophylactic treatment, kidney section Fig 5.1(e) shows significantly reduced tubular vacuolization and crystals deposition compared to the diseased group due to the intake of drug whereas the liver section Fig 5.1(f) shows a central venular congestion.

The curative effect study shows that there is a reduction on number of crystals in the kidney section as shown in Fig 5.1 (g) and a mild liver degeneration is being detected in the curative treated liver section. The pathological observations in the liver and kidney show the efficacy of the extract in restoring the normal function of liver and kidney, which can be one of the signs in management of hyperoxaluria and urolithiasis. The significant contribution of the bioactive compounds from the plant can be the sole reason for reduction in the disease progression (T. Hayashi et al., 1993).
Fig 5.1 The microscopic images of the histopathological sections of (a) Group I control kidney section (b) Group II control liver section (c) Group II ethylene induced kidney section with crystals marked (d) Group II diseased liver section (e) Group III prophylactic drug effect kidney section (f) Group III liver section (g) Group IV curative effect of drug tested kidney section (h) Group IV liver section.

5.4 CONCLUSION

The results suggest that *Scoparia Dulcis*, an Indian Ayurvedic plant can dissolve the urinary crystal and significantly reduce the risk factors of stone formation. The results obtained from the *in vitro* studies were positive enough to check on the drug’s effect on animal models. The *in vivo* study was proved to be successful by analysing the urinary and serum parameters of the Wistar rats and more specifically with the histopathology results. The rats offered with prophylactic and curative therapy showed notable improvement and it was well supported by the histopathology reports.
More than the drug effect in reducing the urolithiasis, the effect of drug in other organs like liver, kidney was also tested and proved that the normal dosage of the drug will not result in a high damage of other organs. Hence, the combined study of the *in vitro* and *in vivo* was able to exhibit that the plant extract and its bioactive constituents have the ability to reduce both struvite and calcium oxalate crystallization. Further studies on bioactive compound isolation and its mechanism of action may help in formulation of a potential anti-urolithic drug.