PROTECTIVE EFFECT OF SPIRULINA ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS
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ABSTRACT
The aim of our present study is to assess the effects of spirulina as a preventive agent in experimentally induced urolithiasis and blood samples were collected from the rats of all the groups at the end of the experiment. The results were analyzed statistically by using one way ANOVA followed by Tukey’s test.

INTRODUCTION
Urinary stone disease is a common disorder found to be increased in western societies for the last five decades. Kidney stone formation is a complex process and it results as a cascade of events, including crystal nucleation, growth and aggregation, crystal retention within the renal tubules. Usually kidney stones are yellow or brown colour with a smooth or gaggled structure. Some common type of kidney stones are calcium oxalate, calcium phosphate, struvite, uric acid and cystine and among these the most common form of kidney stone in humans as well as rats is calcium stones. Approximately 12% of the world population are estimated to be affected by urinary stone disease with a recurrence rate of 70-81% in male and 47-60% in female.

Extra corporeal shock-wave lithotripsy, percutaneous nephrolithotomy, ureteroscopy and nephrolithotomy are the surgical treatment used to eliminate the kidney stones. Among these treatments, extracorporeal shock wave lithotripsy (ESWL) become the standard procedure in eliminating kidney stones. However, ESWL may also cause acute renal injury, a decrease in renal function and also an increase in stone reoccurrence.

A number of non steroidal, anti inflammatory drugs like ibuprofen or narcotics are prescribed to remove the kidney stones all over the world but causes many side effects. Hence, therefore an alternative method was focused to cure lithosis by using medicinal plants or phytotherapy. A number of medicinal plants have been used for the treatment of kidney stone in many parts of the world. Jasmine auriculatum valth, helianthus annulus linn, hibiscus sabdarifaratlinn, hemiaria hirsuta and cynodon dactylon. Spirulina reffered as cyanobacteria or blue green algae is a gram negative bacteria with soft cell wall, consist of complex sugars and protein. It can be rod or disc shaped. Spirulina are photosynthetic with a photosynthetic pigment called phycocyanin, hence autotrophic in nature.

In the present study, the objective was to investigate to validate the antinephrolithic activity of spirulina extract in ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS
Chemicals
All the chemicals and reagents were purchased from S.D fine chemical (mumbai, india). All these were of analytical grade.

Animals
Male albino rats weighing between 150-200gm were selected for antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (25±2°C) and maintained under controlled conditions of 12 hours day and 12 hours night cycle. All the animals were fed with a standard pellets and allowed free access to water.

Experimental protocol
Twenty four animals were randomly divided into four groups containing six animals per group. Each group underwent a different treatment methods for 6 weeks.

Group I: Normal rats feed with normal feed and water.
Group II: Normal rats received 0.75% ethylene glycol (EG) in drinking water alone for first 3 Weeks, followed by drinking water for next 3 weeks.
Group III: Normal rats received 0.75% ethylene glycol (EG) in drinking water for 3 weeks and Spirulina solution (20 mg/kg of body weight /day) for next 3 weeks.
Group IV: Normal rats treated as group1 for first 3 weeks and feed with spirulina solution (20 mg/kg of body weight /day) for next 3 weeks.

Spirulina preparation
The air dried spirulina was mixed under cooling conditions with sterile distilled water and used.

Biochemical analysis
All the treatments were done once daily by orally for 6 weeks. After 6 weeks, the blood samples were collected from the rats of all the groups at the end of the experiment. The results were analyzed statistically by using one way ANOVA followed by Tukey’s test.

In the present study, the objective was to investigate and to validate the antinephrolithic activity of spirulina extract in ethylene glycol induced urolithiasis in rats.
In this study, the ethylene glycol is used to induce the renal tubule due to deposition of calcium oxalate, there is an obstruction in the urine flow in urinary system. Because of this reason, the glomerular filtration rate (GFR) also decreases. Due to this, the wasteproducts, particularly nitrogenous substances such as urea, creatinine and uric acid gets accumulated in blood. It has been already reported that the kidneys are the principle target organ for ethylene toxicity and hence administration of ethylene glycol for 3 weeks resulted in a increased calcium, potassium, sodium and chloride and deposition of crystals in kidney. In addition, the recent study indicated that the significant decrease in phosphorus level in serum promotes the crystal formation in kidney as calcium oxalate. (Group II, Table 1). Treatment with spirulina decreases the calcium, potassium, sodium and chloride levels (Group IV, Table 1). But the level of phosphorus was increased by the administration of the extract.

In urolithiasis, non protein nitrogenous (NPN) substances such as creatinine and urea gets accumulated in the blood and hence the increased concentration in blood of ethylene glycol induced rats (Group II, Table 2). This proves that the ethylene glycol causes renal tubular damage and decreases the glomular filtration rate. The group III suggests that spirulina treated rats brings a significant decrease in the creatinine and urea level near to the normal, but, it does not become normal. These results hastens that spirulina dissolves the preformed stones and prevents the formation of new stones in urinary system in greater extent.

In conclusion, the presented data indicate that administration of ethylene glycol induced lithiasis and also the spirulina extract also seems to have greater preventive effect on induced kidney stones.

ACKNOWLEDGEMENT
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REFERENCES
TABLE 1: EFFECT OF SPIRULINA ON THE LEVEL OF SERUM SODIUM, POTASSIUM, CHLORIDE, CALCIUM AND PHOSPHORUS IN UROLITHIASIS INDUCED RATS

<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>Sodium (mEq/L)</td>
<td>148.72 ± 4.32</td>
<td>160.34 ± 1.94***</td>
<td>157.55 ± 0.52</td>
<td>152.08 ± 3.37</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>6.07 ± 0.24</td>
<td>11.42 ± 0.89</td>
<td>7.73 ± 0.56***</td>
<td>6.00 ± 0.43</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>102.42 ± 0.30</td>
<td>109.54 ± 0.78***</td>
<td>107.91 ± 0.60</td>
<td>101.89 ± 2.63</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.49 ± 0.63</td>
<td>15.50 ± 0.43</td>
<td>11.93 ± 0.35***</td>
<td>10.11 ± 0.57</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>6.10 ± 0.33</td>
<td>2.67 ± 0.31</td>
<td>8.61 ± 0.96*</td>
<td>7.68 ± 0.96</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.D (n=6 animals / group), analysed by one way analysis of variance test (ANOVA) followed by Bonferroni multiple comparison test.

*p < 0.001 statistically significant, **p < 0.01 statistically significant, ***p < 0.05 statistically significant.

*a' comparison are made with Group I.

TABLE 2: EFFECT OF SPIRULINA ON SERUM CREATININE AND UREA LEVELS IN ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

<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>Creatinine</td>
<td>0.42 ± 0.78</td>
<td>1.21 ± 0.23</td>
<td>0.70± 0.04***</td>
<td>0.50± 0.05</td>
</tr>
<tr>
<td>Urea</td>
<td>19.86 ± 0.62</td>
<td>26.39± 0.84</td>
<td>22.95± 1.03***</td>
<td>19.84± 0.85**</td>
</tr>
</tbody>
</table>

All values are mean ± S.D (n=6 animals / group), analysed by one way analysis of variance test (ANOVA) followed by Bonferroni multiple comparison test.

Creatinine and urea were expressed in mg/dl.

*p < 0.001 statistically significant, **p < 0.01 statistically significant, ***p < 0.05 statistically significant.

*a' comparison are made with Group I, 'b' comparison are made with Group II, 'c' comparison are made with Group III.
Figure 1 shows the level of sodium, potassium, chloride, calcium, and phosphorus in serum of normal, ethylene glycol-induced, pretreated (spirulina and ethylene glycol), and spirulina-only rats of four experimental groups.

Figure 2 illustrates the level of serum creatinine in four experimental group animals: normal, ethylene glycol-induced, spirulina and ethylene glycol-treated, and spirulina-only rats.

Figure 3 depicts the level of serum urea in normal, ethylene glycol-induced, spirulina and ethylene glycol-treated, and spirulina-only rats.
Figure 4a, b, c, d represents the histopathology of normal, ethylene glycol induced, spirulina only and pretreated (spirulina and ethylene glycol) rats.

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