1. Introduction

Diuretics play a significant role as adjuvant therapy in certain conditions such as, Edema CHF, Hypertension, obesity, and are commonly being prescribed by the physicians in aforementioned conditions, but the major problem in using these agents is some undesired effect on the body as hypokalaemia, hypomagnesaemia, hypercholesterolaemia, hyperglycaemia with aggravation of diabetes as well as the nonketotic hyperglycaemic syndrome. Diuretic also causes adverse effects on quality of life which includes impotence, fatigue and weakness (Wright et al., 2007; Memory Elvin-Lewis 2001; Freis D Edward 2001). So keeping in view of the above, recently efforts have been made to identify some diuretic agents from the natural source which can achieve the same propose without making unwanted side effects (Bose et al., 2006) Many of the plants from the traditional system of medicine including Unani Medicine which are being used as diuretic since a longtime was scientifically investigated for their diuretic activity (Afzal et al., 2004; Krishna et al 2006) These studies revealed that the diuretic agent from the natural source can be a safeguard and better option to produce the promising effect in comparison to modern diuretic agents in the present study therefore, an important drug of Unani medicine widely acclaimed to be effective in various urogenital conditions.
disorders, viz. Kabab chini (KC) was selected to study its diuretic effect in view of the various pharmacological actions that it has been described to possess by Unani physician such as Mudirre baul (diuretic), Mufattit wa Mukhrije Hasat (liothrotropic) Dafeye taffun (antiseptic), Muqawwiye Kulyah (Kidney tonic), Mohafize Kulyah (nephroprotective) etc. ( Ibn Sina, 1906; Razi, 1967; Ibn-e-Baitar, 2003; Ghani 1921; Dymock, 2005). It is reported to possess diuretic, anti-inflammatory, tonic, antileishmanial, antimicrobial, antioxidant, antileukemic, antimicrobial and nephroprotective activities (Sumathykutty et al., 1999; Choi et al., 2003; Yam J et al., 2008; Hardik et al., 2007; Silva et al., 2007; Aqil et al., 2006; Karthikeyan et al., 2003; Tanjela et al., 1991 zaid et al 2012).
In view of the above, therefore, the present study was designed to evaluate scientifically the diuretic activity of Kabab chini (Piper cubea) in albino rats.

2. Materials and Methods
The present study was undertaken in the department of Ilmul Advia, National Institute of Unani Medicine, Bangalore. Before starting the experiment, the research protocol was submitted for ethical clearance. The Institutional Animal Ethics Committee (IAEC) of National Institute of Unani Medicine, Bangalore approved the protocol for the study. (Registration number: 953/C/06/CPCSEA)

Method of preparation, dosage and mode of administration of test drug
The dried berries of KC (Piper cubea) were provided by the pharmacy of National Institute of Unani Medicine, Bangalore. The test drug was properly identified and authenticated by Dr. Siddamallayya N, of Regional research institute (Ay.) Bangalore vide Ref. No. RRI/BNG/SMP/Drug Authentication /356 and was powdered finely with the help of an electric grinder. The dose of the test drug for the rats was calculated by multiplying the human therapeutic dose7 gm described in Unani literature, by the conversion factor of (Freirich et al 1968). The dose thus calculated for experimental study was found to be 800 mg at which the test drug was studied. However, to study the dose dependent effect of the test drug another dose was also employed by increasing the calculated dose by 50% which was found to be 1200 mg. 5 gm of gum acacia was taken into 100 ml of distilled water. It was shaken vigorously for quite some times to make a homogenous suspension. This suspension was divided into two equal parts. One part was mixed with 8.5 gm of KC powder while the other with 12.5 gm of powder. Both the samples were mixed and shaken well to get a suspension of uniform distribution. The test drugs were prepared a fresh every time before the administration to the animals.

Experimental animals
Healthy adult albino rats of Wistar strain weighing 150-200 gm were used in the study. Animals were maintained on standard diet and water ad libitum unless stated otherwise, and housed in clean polypropylene cages at room temperature (25± 2°C) with a 12h light: 12h dark cycle.

Treatment schedule
The animal model guidelines used for the verification of the diuretic activity was the method described by (Lipschitz et al., 1943) modified by (Kau et al., 1984). This test has been considered as a standard method and it has been widely used to assess diuretic activity of both natural and synthetic potentially diuretic drugs. Each animal then administered 15 ml of isotonic saline to impose a uniform liquid load. The rats were grouped in to 4 each of six animals and they were fasted and deprived of food and water for 18 hours prior to the experiment. The group A, Plain control only administered 0, 9%NaCl, 25ml/kg. The group B administered standard drug furosemide in the dose of 20mg/kg BW and while the group C and D received the test drug in the dose of 800 mg,and 1200 mg respectively.
It is considered that diuretic action of powdered drug generally for 6 to 8h which leads us to select 5h as experimental time termination. The Na and K level was quantified by the method of flame photometer.

Statistical Analysis
Parameters mentioned above were assessed in all the groups and the findings were expressed as Mean ± SEM. The different values determined, were compared with each other and comparison was made by using statistical test of ANOVA one way with Dunnett’s multiple pair comparison test. The difference of mean was considered significant at p value of 0.05 or less.

3. Results and Discussion

Diuretic activity
Effect on urine volume
Results are shown in the table 1. The test drug Kabab chini (Piper cubea) at a dose of 800 mg/kg BW show marked diuresis during the 5 h of the test (5.37±1.43 ml versus control 0.5±0.1.36ml, whereas in case of standard the volume was found to be 4.2±1.16 ml; P ≤ 0.05). However dose dependent diuretic effect of test drug at a dose of 1200mg is relatively low as compared to the group A as it was found 3.35 ±1.74ml

Effect on urinary electrolyte excretion
The effect of single doses of furosemide (20 mg) and test drug Kabab chini (Piper cubeba) at a dose of 800 mg/kg BW on electrolyte (Na⁺, K⁺ and Cl⁻) excretion in the 5 h urine in presented in Table 1. The test drug enhanced the excretion of the electrolytes (P < 0.05). The Na⁺ ion concentration in case of test Drug in group A was 381±3263 ppm while in group B was 351±321 against control i.e. 320±256. K⁺ ion concentration of test drug was 300±367 in group A while in group B it was 276±340 ppm with the concentration of control 240±517ppm. Similarly the Cl⁻ ion concentration of test drug treated group A and B was 290±0.392 ppm and 260±0.230 ppm respectively as well as for the control was 230±0.312.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>Total volume after 5h</th>
<th>Total Na⁺ (ppm)</th>
<th>Total K⁺ (ppm)</th>
<th>Total Cl⁻ (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain control</td>
<td>Normal Saline</td>
<td>20 ml</td>
<td>0.5±0.1.36</td>
<td>320±256</td>
<td>240±517</td>
<td>230±0.312</td>
</tr>
<tr>
<td>Standard Control</td>
<td>Furosamide</td>
<td>20 mg</td>
<td>4.2±1.16</td>
<td>361±327</td>
<td>280±318</td>
<td>247±0.519</td>
</tr>
<tr>
<td>Test Drug A</td>
<td>Piper cubeba</td>
<td>800 mg</td>
<td>5.37±1.43</td>
<td>381±326</td>
<td>300±367</td>
<td>290±0.392</td>
</tr>
<tr>
<td>Test Drug B</td>
<td>Piper cubeba</td>
<td>1200 mg</td>
<td>3.35 ±1.74</td>
<td>351±321</td>
<td>276±340</td>
<td>260±0.230</td>
</tr>
</tbody>
</table>

Discussion

According to ethnopharmacological description of Unani physicians the Kabab chini (Piper cubeba) is a drug of choice for urogenital disorders but to the best of our knowledge, no previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant. In recent period diuretic agents from herbal resources gained popularity as they are devoid of undesirable effect and provide most safe and convenient therapy. Keeping in view of above the test drug was selected to evaluate its therapeutic effect as safe and effective diuretic agent. Cubeb Berry is generally a stimulant, with a special effect on the urinary organs and it is said to produce an augmented flow of urine, while it’s antibacterial properties further help to relieve chronic inflammation of the bladder. The findings of the study indicated a significant diuretic effect of Kabab chini comparable of standard drug furosemide as test drug Kabab chini (Piper cubeba) at a dose of 800 mg/kg BW show marked diuresis during the 5 h of the test (5.37±1.43 ml versus control 0.5±0.1.36ml, whereas in case of standard the volume was found to be 4.2±1.16 ml; P < 0.05). How ever there was no significant increase in diuresis produced by the the test drug on increased dose of test drug at 1200mg /kg BW as it was found 3.35 ±1.74ml that was comparatively lower than group A .the maximum diuretic activity was produced by the Kabab chini (Piper cubeba) at the dose of 800 mg / kg BW.
The intercomparision of the group showed that the test drug has marked diuretic effect at the dose of 800mg rather than 1200 mg and the urine volume and electrolyte concentration which suggested the possible mechanism of action are comparatively higher than standard drug. These data suggest that the test drug at the lower dose has marked diuretic effect in comparison to high dose. The increase in the ratio of concentration of excreted sodium and potassium ions indicated that the test drug increased sodium ion excretion to a greater extent than potassium, which is a very essential quality of a good diuretic with lesser the active principles responsible for the diuretic effects of the plant have not yet been elucidated. The effect may be produced by stimulation of regional blood flow or initial vasodilation (Stanic et al., 1993) or by producing inhibition of tubular reabsorption of water and anions (Pantoja et al., 1993, the result in both cases being diuresis. However, the contribution of polyphenolic compounds to diuretic effect cannot be ruled out. Further studies like isolation and characterization of diuretic principle from the test drug is needed to understand and confirm the exact mechanism of action.

4. Conclusion
The test drug increased urine volume significantly and also potentiate excretion of Na+ in urine output, thus the results obtained in this study provides a quantitative basis to validate the claim of Unani Physicians that the Kabab chini (Piper cubeba) can be use as safe and effective diuretic agent.

5. References