EFFECT OF *Eclipta alba* ON URINARY VOLUME AND ELECTROLYTE EXCRETION IN ALBINO RATS

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**ABSTRACT**

**Introduction:** *Eclipta alba* Hassk (Bhringaraj) grown widely in tropical countries, recently known to have diuretic and anti-hypertensive effect in the tribal community.

**Objectives:** Since the report has shown new dimension to one important clinical use and paucity of data available in this regard, the present work is undertaken to study the above mentioned effects in a scientific manner.

**Materials & methods:** Albino wistar rats (100-200gms) deprived of food for 15hrs divided into seven groups of six in each and put in metabolic cages after hydration by normal saline for 24hrs. Ethanolic extract of *Eclipta alba* (EEEA) was administered in 50, 100, 200 and 400mg/kg doses per oral. Urinary volume, total Na⁺, K⁺, Cl⁻ concentration was estimated at 5th hr & 24th hr and compared with control (saline treated) group. Furosemide (25mg/kg P.O) was taken as the standard.

**Results:** EEEA was found to increase the urinary volume of the 5th hr and 24th hr sample in a dose dependent manner. Na⁺ & Cl⁻ excretion also significantly increased in 200&400 mg/kg doses. Synergistic Effect was not seen with furosemide.

**Conclusion:** EEEA was found to posses’s diuretic activity.

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Introduction:

Diuretics are the pharmacological agents which stimulate rate of urine flow, sodium excretion and modify the volume and composition of body fluids in different pathophysiological condition of body. In many life threatening disease conditions such as congestive heart failure, cirrhosis, renal failure, hypertension, pregnancy toxaemia and nephritic syndrome, the drug-induced diuresis proved as an effective treatment option, which increases patient compliances. [1,2]

Recently, its diuretic and antihypertensive potentiality has been revealed in folklore use of the same which has shown new dimensions to therapeutics. However, paucity of data is available in this regard. Herbal products are cost effective, easily available and safe for long term use. In general diuretics are instructed for long term use; in this context the natural drug provides a safer pharmacotherapy. [3]

Eclipta alba Hassk (Bhringaraja, Fam: Compositae) is a perennial shrub grown widely in the moist tropical countries.[4] It is reported to have anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective, expectorant properties and useful in disease of skin, spleen, stomatitis, toothache, hemicranias as well as vertigo. With this background the present work was undertaken to investigate the diuretic effect of Eclipta alba in a scientific manner.[5,6,7]

Materials & methods:

Plant collection and authentication:

Leaves of Eclipta alba was purchased from the office of Range Officer, Padampur Forest Range, Nrisinghanath, district Bargarh, Odisha & authenticated by the faculty of department of Botany, GM college, Sambalpur.

Preparation of plant extracts:

The whole plant material was dried under sun & was mechanically reduced to moderate coarse powder and stored in an air tight container. The moderate coarse powder was subjected to successive extraction using different solvents in increasing order of polarity using Soxhlet Apparatus of 500 & 1000 ml (Petroleum ether, Chloroform, Ethyl acetate, ethanol & Water). All the respective solvents were recovered under reduced pressure with the help of rotary evaporator.

Acute toxicity study

Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals (albino rats), animals were kept for observation of behavioral change and death up to 72h.

Drugs:

From a pilot study it was indicated that Ethanolic extract of Eclipta alba (EEEA) was the most effective among all other extract. Furosemide was obtained from M/S Hoechst Pharmaceuticals ltd., Mumbai in pure powder form.

Evaluation of Diuretic Activity:

Male wistar albino rats (150-200 gms) were acclimatized to standard laboratory conditions for 15 days. The animals were provided standard rat feed & water ad libitum. Methods of Liptschiz et al was followed for evaluation of diuretic activity. Food but not water was withdrawn 15 hrs prior to the experiment. At the end of 15 hrs, on the day of experiment, all the rats were hydrated with normal saline 20 ml/kg administered orally through a pediatric nasogastric tube. The rats were arranged into seven groups of six in each & the study protocol was designed as depicted in the table 1. [8,9]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment Dose in mg/kg(P.O.)</th>
<th>Mode Of Administration</th>
<th>Nature Of Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NS(20ml/kg)</td>
<td>Through paediatric nasogastric tube</td>
<td>Control</td>
</tr>
<tr>
<td>2</td>
<td>FUROSEMIDE(25)</td>
<td>Dissolved in 1 ml NS followed by 20 ml/kg NS</td>
<td>Standard drug</td>
</tr>
<tr>
<td>3</td>
<td>EEEA(50)</td>
<td>-do-</td>
<td>Test drug</td>
</tr>
<tr>
<td>4</td>
<td>EEEA(100)</td>
<td>-do-</td>
<td>-do-</td>
</tr>
<tr>
<td>5</td>
<td>EEEA(200)</td>
<td>-do-</td>
<td>-do-</td>
</tr>
<tr>
<td>6</td>
<td>EEEA(400)</td>
<td>-do-</td>
<td>-do-</td>
</tr>
<tr>
<td>7</td>
<td>FUROSEMIDE(25mg/kg)+</td>
<td>-do-</td>
<td>Combination of low dose test drug with standard drug</td>
</tr>
<tr>
<td></td>
<td>EEEA(100mg/kg)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1 : Protocol of the study (Approved by IAEC)
Immediately after hydration and treatment the animals were kept in the metabolic cages (3 per one) provided with a wire mesh bottom & funnel with stainless steel sieves to retain faeces and allow the urine to pass. Few drops of toluene were added to each of the collecting cylinder as a preservative to prevent evaporation of urine, since 5th hr & 24th hr urine collection was necessary for the study. The cumulative volume of urine excreted was recorded every hour up to 5th hr & again after 24th hr. The electrolyte concentration of cations (Na⁺, K⁺) was estimated by Ecolyte analyzer & that of anion (Cl⁻) was estimated by end point titration. Data were analyzed by one way ANOVA followed by Dennett’s multiple comparison test.

**Observation & results:**

EEEA 5th hrs after its administration (table 2) shows increase in urinary volume which was significant with 200mg/kg & 400mg/kg doses. Excretion Na⁺ & Cl⁻ followed similar pattern with same doses & chloride excretion were highly significant with two higher dosages. K⁺ excretion were not significant with all the doses tested. Analysis of Na⁺ / K⁺ ratio reveals definite natriuretic effect. The diuretic index was found to be more than that of Furosemide treated group in two high doses of EEEA. Cl⁻ / Na⁺ + K⁺ ratio suggest no evidence of carbonic anhydrase inhibitory activity. The results are comparable to that of Furosemide. Combination of 100mg/kg EEEA with Furosemide 25mg/kg did not exhibit any additive or synergistic effect except chloride excretion which was highly significant.

**TABLE 2: Diuretic activity of EEEA in albino rats 5hr after administration**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (PO)</th>
<th>Cumulative urine volume in ml</th>
<th>Total Na⁺ (mEq / lt)</th>
<th>Total K⁺ (mEq / lt)</th>
<th>Total Cl⁻ (mEq / lt)</th>
<th>Na⁺/K⁺ ratio</th>
<th>Diuretic index</th>
<th>Cl⁻ / Na⁺+K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>11 ± 2.33</td>
<td>155±11.9</td>
<td>65.75± 11.39</td>
<td>128.17 ± 8.545</td>
<td>2.35</td>
<td>-</td>
<td>0.58</td>
</tr>
<tr>
<td>2</td>
<td>Furosemide (25mg/kg)</td>
<td>15.7 ± 0.85</td>
<td>**212.5± 17.96</td>
<td>87±1.505</td>
<td>*222.17± 17.09</td>
<td>2.44</td>
<td>1.42</td>
<td>0.74</td>
</tr>
<tr>
<td>3</td>
<td>EEEA (50 mg/kg)</td>
<td>7.5 ± 1.255</td>
<td>142.5±6.29</td>
<td>74.25±.965</td>
<td>153.81 ± 9.865</td>
<td>1.91</td>
<td>0.68</td>
<td>0.70</td>
</tr>
<tr>
<td>4</td>
<td>EEEA (100 mg/kg)</td>
<td>14.5 ± 1.255</td>
<td>145±20.205</td>
<td>67.25±18.375</td>
<td>178.99 ± 22.93</td>
<td>2.15</td>
<td>1.31</td>
<td>0.84</td>
</tr>
<tr>
<td>5</td>
<td>EEEA (200 mg/kg)</td>
<td>*16 ± 0.815</td>
<td>**187.5±11.085</td>
<td>86.5 ± 4.645</td>
<td>***265.07± 8.545</td>
<td>2.16</td>
<td>1.45</td>
<td>0.96</td>
</tr>
<tr>
<td>6</td>
<td>EEEA (400 mg/kg)</td>
<td>**17 ± 0.575</td>
<td>197.5 ± 6.29</td>
<td>88.5 ± 3.775</td>
<td>***333.25± 35.23</td>
<td>2.23</td>
<td>1.54</td>
<td>1.16</td>
</tr>
<tr>
<td>7</td>
<td>EEEA (100 mg/kg) + Furosemide (25mg/kg)</td>
<td>*15.5 ± 2.25</td>
<td>170 ± 4.08</td>
<td>72 ± 2.71</td>
<td>***247.80± 8.545</td>
<td>2.36</td>
<td>1.40</td>
<td>1.02</td>
</tr>
</tbody>
</table>

* (P < 0.05), ** P < 0.01, *** (P < 0.001)

**Figure 1 : Diuretic activity of EEEA in albino rats 5hr after administration**
Analysis of 24th hr urine sample (table 3) exhibited similar pattern as that of the 5th hr. sample. EEEA exhibited increase in urinary volume, sodium and chloride excretion which were highly significant with 200 & 400 mg/kg doses. K+ excretion was not significant with all the doses tested. The ionic quotient remained parallel with 5th hr values.

**TABLE 3: Diuretic activity of EEEA in albino rats 24hr after administration**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (PO)mg/kg</th>
<th>Cumulative urine volume(ml)</th>
<th>Total Na+ meq/L</th>
<th>Total K+ meq/L</th>
<th>Total Cl- meq/L</th>
<th>Na+/K+ ratio</th>
<th>Diuretic index</th>
<th>Cl- /Na+ + K+</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control( NS)</td>
<td>41±2.33</td>
<td>170±10</td>
<td>72.5±4.785</td>
<td>145.26±4.925</td>
<td>2.34</td>
<td>-</td>
<td>0.59</td>
</tr>
<tr>
<td>2</td>
<td>Furosemide( 25)</td>
<td>***66.25±6.885</td>
<td>**195±8.66</td>
<td>89.5±2.10</td>
<td>***273.44±24.17</td>
<td>2.17</td>
<td>1.61</td>
<td>0.96</td>
</tr>
<tr>
<td>3</td>
<td>EEEA(50)</td>
<td>41.5±2.87</td>
<td>142.5±2.5</td>
<td>72±10.83</td>
<td>187.96±9.865</td>
<td>1.97</td>
<td>1.01</td>
<td>0.87</td>
</tr>
<tr>
<td>4</td>
<td>EEEA(100)</td>
<td>***54.75±3.805</td>
<td>162.5±24.6</td>
<td>76.75±16.75</td>
<td>209.35±20.375</td>
<td>2.11</td>
<td>1.33</td>
<td>0.87</td>
</tr>
<tr>
<td>5</td>
<td>EEEA(200)</td>
<td>***73.5±11.84</td>
<td>180±8.165</td>
<td>78.75±9.455</td>
<td>***290.5±9.865</td>
<td>2.28</td>
<td>1.79</td>
<td>1.12</td>
</tr>
<tr>
<td>6</td>
<td>EEEA(400)</td>
<td>***89±10.15</td>
<td>187.5±7.5</td>
<td>83.75±2.84</td>
<td>***367.43±25.55</td>
<td>2.23</td>
<td>2.17</td>
<td>1.35</td>
</tr>
<tr>
<td>7</td>
<td>EEEA(100)+ Furosemide(25)</td>
<td>***52.5±4.515</td>
<td>147.5±7.5</td>
<td>65.75±2.175</td>
<td>***273.44±3.955</td>
<td>2.24</td>
<td>1.28</td>
<td>1.28</td>
</tr>
</tbody>
</table>

**Discussion:**

Diuretic activity of EEEA 5 hr after its administration was manifested in the form of an increase in urinary volume which was highly significant with 200 and 400 mg/kg doses. Excretion of sodium and chloride followed similar pattern with same doses. Potassium excretion was not significant with all the doses tested which indicated potassium conserving property of the herbal extract. Analysis of Na+/K+ ratio revealed a definite natriuretic effect. (Na+/K+ values greater than 2.0 indicate a favorable natriuretic effect. Ratios greater than 10.0 indicate a potassium sparing effect).[10,11] Therefore, though EEEA showed a potassium conserving potentiality, potassium sparing effect was not observed. The diuretic index found to be more than that of Furosemide in the two higher doses. The value of Cl- / Na+ + K+ ratio varied between 0.70 to 1.16 with graded doses of EEEA suggesting a negative carbonic anhydrase inhibitory activity. The results were comparable to that of Furosemide.

Analysis of 24th hour post dosing urine sample revealed similar results with regards to urinary volume, sodium, chloride and potassium excretion as observed in 5th hr sample that indicates a continuation of the diuretic effect of EEEA up to 24 hours. The ionic quotient remained parallel with the 5th hr values. No mortality, behavioral changes observed in the experimental animals during the whole period of study, which indicate safe and low toxicity of EEEA in long-term use.

From literature survey it is quite evident that diverse herbal product posses’s significant diuretic potentiality of clinical usefulness reported by various workers in different set up of studies. [12, 13, 14] The results of the present study with Eclipta alba is in agreement with the fellow co-workers.

In a pilot study, on patient with mild hypertension (ayurvedic formulation study), observed the diuretic, hypotensive and hypocholesterolemic effects of Eclipta alba.[7] The patients supplemented with Eclipta alba showed a marked increase in urine volume (34%), urinary sodium(24%) and a parallel decrease in blood pressure.

An herbal product usually contains many active components viz. flavonoides, steroid, glycosides, saponins, organic acids etc. which either alone or in combination is responsible for the diuretic activity.[13] No additive or synergistic effect was observed by combined administration of Furosemide & low effective dose (100m g/kg) of EEEA.

**Conclusion:**

The results of the present investigation clearly indicates potential diuretic activity of Eclipta alba Hassk in rats. Hence phytochemical analysis, separation of active ingredients and further investigation in this line is essential to establish its therapeutic benefits.

**Acknowledgment:**

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**Author’s statement Conflict of interest**
I/We, the undersigned author(s) of the manuscript entitled *Effect of eclipta alba on urinary volume and electrolyte excretion in albino rats*, hereby declare that the above manuscript which is submitted for publication in the *The Indo American Journal of Pharmaceutical Research* (IAJPR) is NOT under consideration elsewhere. The manuscript is NOT published already in part or whole (except in the form of abstract) in any journal or magazine for private or public circulation. Each author has participated sufficiently in the work to take public responsibility for appropriate portions of the content. The order of authorships on the byline is a joint decision of all the coauthors. In case the manuscript should be accepted for publication, I have the consent of each author to transfer and assign any and all right, title and interest, including copyright of the article referenced above.

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