The Diuretic Activity of *Ephedra alata* and *Plumbago europaea* in Mice using an Aqueous Extract

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Abstract

Throughout history, mankind used various natural materials as a remedy for the treatment of various diseases and recently witnessing a vastly growing and renewed interest in herbal medicine globally. In Palestinian folk medicine, *Ephedra alata* and *Plumbago europaea* used as diuretics and for treatment of hypertension. This study aimed to evaluate the diuretic and acute toxicity effects of the aqueous extracts for these two plants in mice following oral administration. Aqueous *E. alata* and *P. europaea* extracts (500 mg/kg) were administered orally to adult mice. Urine output and electrolytes were then measured after 4 h of administration and compared with those received furosemide 10 mg/kg (positive control group) and those received normal saline (negative control group). Significant diuresis was noted in those receiving the aqueous extract of *E. alata* (*p < 0.001*), while the *P. europaea* aqueous extract had shown mild diuresis at the end of the forth hour compared to controls. Moreover, both aqueous extracts had an alkaline pH and a mild increase in the electrolyte excretion (Na, K). Our results revealed that *E. alata* aqueous extract has a potential diuretic effect. Further studies are needed to evaluate this diuretic effect in the relief of diseases characterized by volume overload.

**Keywords:** *Ephedra alata, Plumbago europaea, Aqueous extracts, Diuretic activity*
1. Introduction

For centuries, plant kingdom has been a valuable and endless source of therapeutic medicaments, and many of newly discovered drugs are isolated from plants products or their derivatives [1, 2]. However, since natural products derived drugs investigations and isolations were associated with some basic difficulties, most of the pharmaceutical companies have shifted their fundamental focuses toward purified synthetic or semi-synthetic chemical compounds [3, 4]. Unfortunately, the obtained results did not meet the global health expectations as evident in a decreasing number of novel medications reaching the pharmaceutical markets as well as due to the unexpected harmful side effects, contraindications and drug-drug interactions of chemical drugs [1, 5]. For these reasons the global interests moved again toward the natural products based drugs development, even it necessitates broad interdisciplinary research approaches [6]. A common approach is to start pharmacological research with crude plant extracts and subsequently isolate and characterize the ingredients responsible for the pharmacological effect of the extract [7]. Diuretic drugs are used normally to increase the urine output to reform and adjust the normal body composition of fluids. Therefore it’s necessary to relieve some life-threatening diseases such as; hypertension, congestive heart failure, cirrhosis, nephritic syndrome and pregnancy toxemia [8]. Plumbago europaea L., commonly known as leadwort, a perennial plant which is native to the Mediterranean regions and Central Asia. This plant is known to contain plumbagin naphthoquinones, terpenoids, and europetin flavonoid, India, China, and other Asian countries have been used P. europaea as immuno-suppressant, antitumor, antirheumatic, anti-wart and for treatment of dysmenorrhea [9, 10]. It is used for the treatment of edema, inflammations, hepatitis, leprosy, respiratory disorders, toothache, warts, scabies, blisters, and calluses in traditional Palestinian, Jordanian, Turkish and Italian traditional medicines [11-17]. Ephedra alata Decne. (the Arabic name is Alanda, family Ephedraceae) is a perennial herbaceous shrub belonging to the Gnetae plant. The plant origin is Palestine, Saudi Arabia, Algeria, Egypt, Chad, Iraq, Iran, Tunisia, Lebanon, Jordan, Morocco, Syria, Libya, Mali, Mauritania, and Somalia [18]. The stems of E. alata is used in the folk medicine for treating urinary tract, cardiovascular, respiratory and digestive systems disorders and for treatment of cancer, fungal and bacterial infectious diseases [19-21]. Ephedra alata contains a mixture of flavonoids herbacetin 7-O-(6″-quinylglucoside), herbacetin 8-methyl ether 3-O-glucoside-7-O-rutinoside, viceninII, kaempferol 3-rhamnoside, lucenin III, herbacetin 7-glucoside and quercetin 3-rhamnoside [22]. Meanwhile, Plumbago europaea contains a mixture of flavonoids such as plumbagin, hydroplumbagin-4-O-glucoside and myricetin-3-O-rhamnoside [23]. In fact, various scientific studies have been proved the diuretic effect of flavonoids [24-26]. Therefore, we suppose that these two plants might have a diuretic effect due the presence of this class of biologically active compounds. This study evaluates the diuretic and acute toxicity effects of the aqueous extracts for these two plants in mice a and comparing them to furosemide.

2. Methodology

2.1 Instrumentation and chemicals

Freeze dryer (Mill rock technology, model BT85, China), grinder (Moulinex model, Uno, China), balance (Beco, Germany), hot plate (Labtech, South Korea), filter paper (Whatman no.1, USA), furosemide (Jerusalem Pharmaceutical company, Palestine) and NaCl (Salit, Israel).

2.2 Collection and preparing plant materials

The entire E. alata and P. europaea plants were collected in June 2016 from the mountains of Jenin region of West Bank/Palestine. The plants were identified by the pharmacognosist Dr. Nidal Jaradat. A voucher specimen was
deposited in the Laboratory of Pharmacognosy. Voucher specimen code for the *E. alata* was (Pharm-PCT-904) and (Pharm-PCT-1899) for *P. europaea*. The plants were washed well using distilled water to avoid any contaminations and then dried in the shade at room temperature until all the plant parts became dry. After drying, the plant materials were grounded well using a mechanical blender into a fine powder and transferred into airtight containers with proper labeling for use.

2.3 Preparation of plants dry extracts
The aqueous plants extracts were infused by taking 100 ml of boiling water in a beaker and added to it 10 g of dry plant material then covered and incubated for 30 min. The infusion then was filtered using Whatman filter paper No. 1 and concentrated to 10 ml in a water bath at 45°C (1 ml of this extraction equivalent to 1 g of dry starting material; all doses are expressed in terms of starting material). Extracts were stored at 4°C in the refrigerator less than 1 week. After that the plant extract dried by using a freezing dryer to produce a dry extract. Then 15 mg plant powdered extract was dissolved in 15 ml 0.9% normal saline solution (0.9 mg of solid NaCl was dissolved in 100 ml distilled water to produce 0.9% concentration) to produce a 1mg/ml concentration [27].

2.4 Animal
Male CD-1 mice (weight range: 25-30 g) at the beginning of the study were used in the experiment. They were kept three per cage in the animal house. Mice were acclimatized to the animal facility for 7 days prior to testing under controlled conditions of temperature (22±2°C). Mice were re-used with a minimum 7 days’ interval between drug testing. All experiments were performed during the light portion of the day cycle. All animals were fasted over the night of the experiment. The animals were pretreated with physiological saline (0.9% NaCl) at an oral dose of 0.15 ml/10 g body weight, to impose a uniform water and salt load [28-30]. All studies were approved by An-Najah National University Animal Care and Use Committee, in accordance with guidelines established by the National Research Council.

2.5 Diuretic test
For diuretic effect determination, the male mice were deprived of water for eighteen hours prior to the experiment. The following day the mice groups were given 5 ml/kg of Furosemide orally (40 mg furosemide in 40 ml NaCl to make 1mg/ml concentration solution), normal saline solution, *E. alata*, and *P. europaea* (150µl of each) respectively. Twelve mice were divided into four groups, three mice in each. The first group (serving as control group) was given 150µl normal saline; the second group received 150µl, furosemide (5ml/Kg) in saline; the third group received *E. alata* extract at the doses of (5ml/Kg), and the fourth group received *P. europaea* extract at the doses of (5ml/Kg) in normal saline by using a micropipette. Mice were re-used with a 7 days’ interval between drug testing. Mice were placed in the cages which having a wire mesh fitted with a small container for urine collection. The urine was collected using measuring cylinders up to 4 hours after dosing. During this period, no food or water was available to animals [30]. The volume of each group was collected and observed each hour. Urine was then measured 1, 2, 3, and 4 hrs after dosing. The urine was then filtered and stored at -20°C for electrolyte analyses [29]. In order to compare the effects of the extracts with vehicle and standard on urine excretion, the following parameters were calculated. The urinary excretion independent of the animal weight was calculated as total urinary output divided by total liquid administered (Formula 1). The ratio of urinary excretion in test groups to urinary excretion in the control group was used as a measure of diuretic action of a given dose of extract (Formula 2). A parameter known as diuretic activity was
also calculated through comparing the diuretic action of the extract to that of the standard drug in the test group (Formula 3) [28].

2.6 Measurement of urine pH, Na⁺ and K⁺

After being stored at -20°C, the samples were diluted (1:5 in deionized water) and urine pH, Na⁺ and K⁺ concentrations were measured using ion-selective microelectrodes according to manufacturer’s protocol (Lazar Research Laboratory, Inc, Los Angeles, CA, USA). Total amounts of each electrolyte were quantified for each 4 h sample using the formula: 5×diluted sample concentration (μEq/ml) × total volume (ml) of sample [31].

2.7 Acute Toxicity Testing

The aqueous extracts of E. alata and P. europaea were evaluated for its toxicity in female Swiss albino mice at the age of 6-8 weeks, at a dose of 5000 mg/kg body weight according to OECD guidelines No 425 [32]. The animals were deprived of food for 18 hours with free access to water. Immediately after administration of the extract, the animals were carefully observed continuously for the first 4 hours for any overt signs of toxicity and death and then for the next 24 hour. Thereafter, they were kept under close observation up to 14 days to monitor the presence of any signs of morbidity or mortality. The weight of each animal was recorded at the 1st, 7th, and 14th day of administration to verify any weight change that might have occurred [33].

2.8 Statistical Analysis

Descriptive statistics, including mean ± standard error (SE) and confidence intervals (CI), were used to summarize data. Paired-sample t-test were used to compare between-group differences in mean 4 hours’ urine volume output and concentration of K⁺ and Na⁺ electrolytes in urine. Significant differences were set at P values ≤ 0.05.

2.9. Ethical issues

IRB from An-Najah National University has approved the study

3. Results and discussion

3.1 Acute toxicity study

During 2 weeks of observation, none of the extracts plant produced any visible signs of toxicity up to the dose of 5 g/kg. Signs of toxicity included loss of weight, tremor, paralysis, lethargy, stress or adverse behaviors. In addition, there was also no sign of diarrhea and none of the treated mice died, suggesting the lethal dose (LD₅₀) is greater than 5g/kg.

3.2 Diuretic activity:

The aqueous extract of the whole plant of E. alata showed a significant increase in urine volume (P<0.001) compared to the control group as shown in table 1 and 2, and Figure 1. On the other hand, the aqueous extract of P. europaea revealed a potential diuretic effect. The time course of action of diuresis is depicted in figure 1 and table 2.
Table 1. Effect of aqueous extracts of *E. alata* and *P. europaea* on urine volume in mice within 4 hours

<table>
<thead>
<tr>
<th>Samples</th>
<th>Mean difference in urine output (ml)</th>
<th>95% CIs</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide vs Control</td>
<td>383.5</td>
<td>301.5, 465.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>E. alata</em> vs control</td>
<td>207.0</td>
<td>125.0, 289.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>P. europaea</em> vs control</td>
<td>66.5</td>
<td>-15.6, 148.6</td>
<td>0.111</td>
</tr>
<tr>
<td>Furosemide vs <em>E. alata</em></td>
<td>176.5</td>
<td>94.5, 258.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Furosemide vs <em>P. europaea</em></td>
<td>317.0</td>
<td>235.0, 399.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><em>E. alata</em> vs <em>P. europaea</em></td>
<td>140.5</td>
<td>58.5, 222.6</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

SE indicates Standard Error; CI, confidence interval

Figure 1. The 4 hours’ urine output volumes for four mice groups.

Table 2. Effect of aqueous extracts on 4 h urine volume output in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
<th>Diuretic action</th>
<th>Diuretic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>68 ± 18</td>
<td>166 ± 72</td>
<td>190 ± 80</td>
<td>204 ± 77</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>332 ± 81</td>
<td>390 ± 105</td>
<td>680 ± 136</td>
<td>760 ± 117</td>
<td>3.70</td>
<td>1</td>
</tr>
<tr>
<td>Pumbago</td>
<td>138 ± 60</td>
<td>230 ± 70</td>
<td>244 ± 65</td>
<td>282 ± 58</td>
<td>1.38</td>
<td>0.37</td>
</tr>
<tr>
<td>Ephedra</td>
<td>226 ± 72</td>
<td>300 ± 115</td>
<td>430 ± 150</td>
<td>500 ± 135</td>
<td>2.45</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Data are a mean ± standard error of the mean

3.3 Saluretic activity: effect on electrolyte content of the urine

The urine samples collected over the four hours were analyzed for the electrolyte content (Na+ and K+) and presented in Table 3, 4 and 5. The aqueous extracts of *E. alata* and *P. europaea* tended to increase sodium and potassium loss compared to the control group, but the results were not significant statistically.
Table 3. Urinary electrolyte excretion in mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Na⁺</th>
<th>K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>74.8 ± 4.4</td>
<td>72.9 ± 22.4</td>
</tr>
<tr>
<td>Furasamide</td>
<td>167.7 ± 8.3</td>
<td>95.5 ± 5.8</td>
</tr>
<tr>
<td>Epherdra alata</td>
<td>118.5 ± 35.4</td>
<td>109.3 ± 17.4</td>
</tr>
<tr>
<td>Plumbago europea</td>
<td>55.9 ± 15.5</td>
<td>121.4 ± 8.5</td>
</tr>
</tbody>
</table>

Data are a mean ± standard error of the mean

Table 4. The concentration of Na⁺ electrolytes in the urine of the four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference of mean</th>
<th>SE of the mean difference</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furasamide vs control</td>
<td>92.9</td>
<td>9.4</td>
<td>56.4, 129.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Epherdra alata vs control</td>
<td>43.7</td>
<td>35.7</td>
<td>-195.5, 283.0</td>
<td>0.671</td>
</tr>
<tr>
<td>Plumbago europea vs control</td>
<td>-18.9</td>
<td>16.1</td>
<td>-281.1, 243.3</td>
<td>0.720</td>
</tr>
<tr>
<td>Epherdra alata vs Furasamide</td>
<td>-49.2</td>
<td>36.4</td>
<td>-275.5, 176.6</td>
<td>0.613</td>
</tr>
<tr>
<td>Plumbago europea vs Furasamide</td>
<td>-111.8</td>
<td>17.6</td>
<td>-273.2, 49.6</td>
<td>0.089</td>
</tr>
<tr>
<td>Plumbago europea vs Epherdra alata</td>
<td>-62.6</td>
<td>38.7</td>
<td>-267.7, 142.5</td>
<td>0.494</td>
</tr>
</tbody>
</table>

SE indicates Standard Error; CI, confidence interval

Table 5. Concentrations of K⁺ electrolytes in the urine of the studied four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference of mean</th>
<th>SE of the mean difference</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furasamide vs control</td>
<td>22.7</td>
<td>21.3</td>
<td>-45.6, 90.9</td>
<td>0.720</td>
</tr>
<tr>
<td>Epherdra alata vs control</td>
<td>36.4</td>
<td>21.3</td>
<td>-31.9, 104.6</td>
<td>0.380</td>
</tr>
<tr>
<td>Plumbago europea vs control</td>
<td>48.5</td>
<td>21.3</td>
<td>-19.7, 116.8</td>
<td>0.380</td>
</tr>
<tr>
<td>Epherdra alata vs Furasamide</td>
<td>13.7</td>
<td>21.3</td>
<td>-54.5, 82.0</td>
<td>0.915</td>
</tr>
<tr>
<td>Plumbago europea vs Furasamide</td>
<td>25.9</td>
<td>21.3</td>
<td>-42.4, 94.1</td>
<td>0.636</td>
</tr>
<tr>
<td>Plumbago europea vs Epherdra alata</td>
<td>12.1</td>
<td>21.3</td>
<td>-56.1, 80.4</td>
<td>0.938</td>
</tr>
</tbody>
</table>

SE indicates Standard Error; CI, confidence interval

3.4 Urinary pH

The urine pH measurement of the studied groups showed that the aqueous extracts of E. alata and P. europaea had produced relatively alkaline urine as shown in figure 2. About 80% of the population use herbal medicines in developing countries, mainly, for primary health care due to their better acceptability among humans and lesser side effects. In the last decades, a lot of efforts have been made into researching for traditional plants with therapeutic values [34, 35]. Diuretics are therapeutic agents which utilized to increase the urine output or/and sodium excretion, in order to adjust the composition and volume of body fluids also to eliminate the excess of fluids from tissues. For these reasons, they were used for adjustment and treatment of various conditions, including heart failure, hypertension, renal
failure, liver cirrhosis, lung and kidney diseases [36, 37]. There are a growing number of studies purporting diuretic effects with traditional medicines. These might offer a natural safeguard against the development of certain conditions and be a treatment for some diseases. [38-40]. Aqueous extracts of *E. alata* and *P. europaea* had diuretic activity without any toxic effect at 5 g/kg dose with alkaline pH for both extracts. In fact, the diuretic effect of *E. alata* was much better *P. europaea* and the urine volumes after 4 hours of consumption of these extracts were 500 ± 135 ml and 282 ± 58 ml, respectively in comparison with furosemide 760 ± 117 ml of urine volume. Regarding these results, the *E. alata* aqueous extract had strong diuretic action comparing with furosemide. The present study supports the ethnomedical use of the studied plants for its diuretic effect and based on the pattern of water, sodium and potassium excretions it appears that the plant could possibly have more than one physiological mechanism of action which contributes to the potassium- saving and natriuretic effect especially at the maximal doses. In a study which was conducted by Wubshet H. and Ephrem E. on the diuretic effect of some Ethiopian traditional medicinal plants also this study supported their folkloric use as diuretic agents [41]. Further pharmacological and phytochemical studies required to identify and to isolate the active molecule in the studied plants which were responsible for this effect also additional clinical trial required to evaluate the diuretic action on human as well as *E. alata* aqueous extract used intensively in the Palestinian folk medicine as a diuretic agent.

![Fig. 2 Urinary pH of control, furosemide, E. alata and P. europaea](image)

**Fig. 2** Urinary pH of control, furosemide, *E. alata* and *P. europaea* (Error bars represent 95% confidence intervals)

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