ABSTRACT

Objective: The objective of this study is to test diuretic effect of ethanol extract of P. acidus leaves in female Wistar rats using modification of Lipschitz method.

Methods: The doses of the extract were 22.5, 45 and 90 mg/kg bw. Parameter of diuretic effect was cumulative urine volume during 6 and 24 hours after administration of the extract. Sodium and potassium level in urine were analyzed using atomic absorption spectrophotometry (AAS). The results were analyzed with Student-t test.

Results: The ethanol extracts of P. acidus at doses of 22.5, 45, and 90 mg/kg bw could accelerate the urinary in rats since 60 minutes after oral administration compared to the control and furosemide 3.6 mg/kg bw (P<0.05). The level of sodium on 24 hour urine of the ethanol extracts of P. acidus at doses 22.5 and 45 mg/kg bw significantly higher than control group (P<0.05), but significantly lower than furosemide 3.6 mg/kg bw (P<0.05). The level of potassium on 24 hour urine of the ethanol extracts of P. acidus at doses 45 and 90 mg/kg bw significantly higher than control group (P<0.05), but significantly lower than furosemide 3.6 mg/kg bw (P<0.05).

Conclusion: It can be concluded that the ethanol extract of P. acidus at doses of 22.5 mg/kg bw, 45 mg/kg bw and 90 mg/kg bw had significantly diuretic and saluretic effect compared to control group (P<0.05), but significantly less than furosemid 3.6 mg/kg bw (P<0.05).

Keywords: Phyllanthus acidus, Ceremai, Diuretic, Saluretic, Sodium, Potassium.

INTRODUCTION

One of traditional medications that accepted by WHO is herbal medicines. Herbal medicines usually use in developing countries for primary health care to treat various diseases based on long historical use of the herbal medicines itself. The term herbal medicines include herbs, herbal materials, herbal preparations and finished herbal products, which contain as active ingredients parts of plants, or other plant materials, or combinations [1]. A review of herbal medicine as diuretic by Wright et al. (2007) stated that the most promising herbs are Foeniculum vulgare, Fraxinus excelsior, Hibiscus sabdariffa, Petroselinum sativum, Spargularia purpurea, species from the genuses Cucumis (Cucumis melo and Cucumis trigosus), Equisetum (Equisetum bogotense, Equisetum fluviatile, Equisetum giganteum, Equisetum hiemale var. affine and Equisetum myriochaetum), Lepidium (Lepidium latifolium and Lepidium sativum), Phyllanthus (Phyllanthus amarus, Phyllanthus corcovadensis and Phyllanthus sellowianus) and Sambucus (Sambucus mexicana and Sambucus nigra) [2].

Traditionally several Phyllanthus sp. is used as diuretic. Udupa et al. (2010) have tested the diuretic effect of aqueous extract of Phyllanthus niruri, the result showed the significant increase in the volume of urine and excretion of sodium, potassium and chloride after the extract administered in hydrated albino rats [3]. An evaluation of the diuretic activity of methanolic extracts of aerial parts of P. fraternus showed that the extract increased the volume of urine and ionic concentration of sodium and potassium levels in urine as compared with control group [4]. Diuretic effect of an aqueous extract (5% w/v) of the stem bark of P. sellowianus (400 mg/kg bw) also tested on animals, which showed that after 8 h of the single oral administration, the extract increased a significant in the urinary excretion [5]. Even, Srividiya and Periwal (1995) have observed the potential effect of P. amarus as diuretic, hypotensive, and hypoglycaemic on human subject [6]. Another Phyllanthus sp. that easily found in Indonesia is Phyllanthus acidus Linn. Phyllanthus acidus Linn has been used traditionally to treat inflammation, hypertension, diabetic and hyperlipidemia [7]. One of the mechanisms of antihypertension is diuretic, which work by increasing the excretion of urine from the body as well as the amount of sodium in urine [2], and P. acidus is suspected has diuretic effect. The objective of this study is to test diuretic effect of ethanol extract of P. acidus (L.) Skeels leaves.
Female Wistar rats (150-250g) were used for the diuretic test. The animals were obtained from Center of Life Sciences, Institut Teknologi Bandung, Bandung, Indonesia and housed under standard laboratory conditions. The animals fed with standard rat feed and water ad libitum. Female rats were used in order to give more comprehensive results. Craft et al (2000) has been evaluated the diuretic effect of bremaozocine, pentazocine, and furosemide in females and males rats and the result showed that when data were corrected for individual difference in body weight, furosemide produced nearly equivalent diuresis/kg in females and males rats [9]. Prior the experiment, the animals were fasted for 18 hours except water was given ad libitum. The animals were divided into five groups (five in each). The extract was suspended in 0.5% sodium carboxymethyl cellulose (Na CMC) for oral administration. The doses of the extract were 22.5, 45 and 90 mg/kg bw. Furosemide (3.6 mg/kg bw p.o) in 0.5% Na CMC was used as the reference diuretic agent. In the beginning of the experiment, the animals were given NaCl 0.9% 20 ml/kg bw as loading dose. After 30 minutes, the animals groups were treated as follows:

**Group I (control):** received 0.5% Na CMC orally at a dose of 10 ml/kg bw.

**Group II (standard):** received furosemide orally at a dose of 3.6 mg/kg bw.

**Group III (test 1):** received ethanol extract of P. acidus orally at a dose of 22.5 mg/kg bw.

**Group IV (test 2):** received ethanol extract of P. acidus orally at a dose of 45 mg/kg bw.

**Group IV (test 3):** received ethanol extract of P. acidus orally at a dose of 90 mg/kg bw.

After treatment, the animals were kept in a metabolic cage. The urine was collected and measured every 30 minutes for 6 hours and 24 hours after the dose was administered. Parameter of diuretic effect was total urine output during 6 and 24 hours after administration of the extract and the percentage of total urine output compared to the volume of NaCl 0.9% which is given as loading dose. The diuretic effect is stated as the positive result if the percent of total urine output is higher than 80%. Sodium and potassium level in urine was analyzed using atomic absorption spectrophotometer (AAS).

**Statistical analysis**

All the values expressed are Mean ± S. E. M. Statistical evaluations were performed by Student’s t-test at the 95% confidence level using an SPSS program for Windows 17.0. Values of p < 0.05 were considered significant.

**RESULTS**

**Phytochemical analysis**

The ethanol extract of P. acidus leaves contain flavonoids, triterpene, monoterene and sesquiterpene as shown in table 1.

**Diuretic effect**

Diuretic effect of the ethanol extract of P. acidus leaves is characterized using urinary output (volume of urine) and ionic concentration of electrolytes (sodium and potassium).

### Effect on urinary output

Urinary profile every 30 minutes for 6 hours after administered of the extract can be seen in fig. 1, while total urine output after 6 hours and 24 hours can be seen in table 2.

**Table 1: It shows phytochemical analysis in ethanol extract of P. acidus leaves**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>–</td>
</tr>
<tr>
<td>Tannin</td>
<td>–</td>
</tr>
<tr>
<td>Saponins</td>
<td>–</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>–</td>
</tr>
<tr>
<td>Triterpene</td>
<td>+</td>
</tr>
<tr>
<td>Monoterpene and sesquiterpene</td>
<td>(+) = present; (–) = absent</td>
</tr>
</tbody>
</table>

**Table 2: It shows total urine output in rats at 6 and 24 hours after oral administration of ethanol extract of P. acidus at doses of 22.5, 45 and 90 mg/kg bw, and vehicle and reference drug furosemide 3.6 mg/kg bw**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total urine output (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 hours</td>
</tr>
<tr>
<td>Control</td>
<td>2.45±1.04</td>
</tr>
<tr>
<td>Furosemide 3.6 mg/kg bw</td>
<td>3.23±0.58</td>
</tr>
<tr>
<td>Ethanol extract of P. acidus 22.5 mg/kg bw</td>
<td>4.93±1.16*</td>
</tr>
<tr>
<td>Ethanol extract of P. acidus 45 mg/kg bw</td>
<td>5.96±2.04*</td>
</tr>
<tr>
<td>Ethanol extract of P. acidus 90 mg/kg bw</td>
<td>5.49±3.23</td>
</tr>
</tbody>
</table>

n = 5, *P<0.05, compared to control group using Student t-test
significantly higher than control group (P<0.05), and also higher than a reference drug furosemide 3.6 mg/kg bw. However, total urine output at 24 hours after oral administration of the ethanol extract of P. acidus leaves at doses of 22.5, 45, and 90 mg/kg bw significantly higher than control group (P<0.05), but slightly lower than the furosemide 3.6 mg/kg bw.

**Effect on urinary electrolytes**

Sodium and potassium level on 24 hours urine is shown in table 3.

The result showed that the ethanol extract of P. acidus leaves at doses of 22.5, 45, and 90 mg/kg bw could increase sodium and potassium level on 24 hour urine. The level of sodium of the ethanol extracts of P. acidus leaves at doses 22.5, 45, and 90 mg/kg bw significantly higher than control group (P<0.05), but significantly lower than furosemide 3.6 mg/kg bw (P<0.05). Further study of the component that involved in diuretic effect and the exact site of action are needed.

**CONCLUSION**

It can be concluded that the ethanol extract of P. acidus leaves at doses of 22.5, 45, and 90 mg/kg bw had significantly diuretic and saluretic effect compared to control group (P<0.05), but significantly less than furosemide 3.6 mg/kg bw (P<0.05). The authors declare no conflict of competing interest.

**ACKNOWLEDGEMENT**

The authors thank Ministry of Higher Education of Republic of Indonesia for funding this research.

**REFERENCES**


