Diuretic Activity of Roots from Carica papaya L. and Ananas comosus L.

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ABSTRACT

Water extracts of Carica papaya and Ananas comosus extracts were administered orally at doses of 5 and 10 mg/kg to Sprague-Dawley rats. Two other groups were given commercial diuretics, furosemide and hydrochlorothiazide at 10 mg/kg. Urine volume, urine pH, urine density and urine electrolytes were determined every hour for 4 hours. Blood was taken to determine the serum level of glucose, albumin, blood urea nitrogen (BUN) and creatinine. Both, C. papaya and A. comosus extracts exhibited moderate to potent diuretic activity. C. papaya extract increased the 4-hour urine volume when administered at both 5 and 10 mg/kg. A. comosus extracts increased urine volume only at 10 mg/kg dose. Na+ and Cl− elimination was unchanged for the whole period of study. However, the 4-hour uriection of K+ were significantly increased for both plant extracts. Both plant extracts induced increment of serum BUN and creatinine level significantly when compared to controls (p<0.05) but these levels were still within the normal range. Blood glucose level and albumin were not significantly for both plant extract. In conclusion, both plants investigated had diuretic activity and roots of C. papaya activity were statistically similar potency to the activity of furosemide and hydrochlorothiazide. However, care must be taken when using these herbs as increased urinary K+ being excreted and marginal increase of serum BUN and creatinine were detected.

Keywords: Word diuretic, electrolytes, Carica papaya, Ananas comosus.

INTRODUCTION

Medicinal plants are used for many various diseases. Plants commonly used for traditional treatment of renal diseases have been reported to show significant diuretic activity. Herbal plants may also be a useful tool in the treatment of hypertension. These previous studies emphasized the application of plants as a diuretic agent in treatment of dysuria and hypertension. Currently, commercial synthetic diuretic drugs are prescribed in the treatment hypertension, congestive heart failure and other cardiovascular diseases.

Carica papaya or ‘betik’ in Malay is a pantropical, small, soft-wooded, usually unbranched tree, 7 to 8 meter tall, lactiferous, fast growing and short lived. Large glabrous palmatifid and palmatinerved leaves (30-60 cm) and flowers are aromatic. Fruits are large ovoid and smooth, green while young and yellow-greenish or orange when ripe. Ananas comosus or ‘nanas’ in Malay is perennial, stout herbaceous, sometimes spinescent succulent and up to 1 m tall. It produces numerous, elongated, finely toothed, long, sword-like, arranged in a tight spiral around a short stem, edges very sharply dentate to nearly entire, often variegated, or red or brown streaked. Stems are erect and long. Flowers are purplish-blue, timorous, and progressive toward apex of stem, with oldest flowers at base of inflorescence. A fruit is cone-like, spirally gathered into a large ovoid fleshy, edible mass crowned by persistent bracts.

Even though these plants have been used in traditional medicine for centuries, the mechanism of actions is yet to be discovered. This present study was aimed to evaluate the diuretic activity of the aqueous extract of the Carica papaya and Ananas comosus and elucidate the possible mechanism of action of diuretic activity of plant extracts.

MATERIALS AND METHODS

Plants materials and Preparation of the extracts

The plants were collected from Sepang District, Selangor, Malaysia and were verified. Samples were deposited in the Phytomedicinal Herbarium Institute of Bioscience, Universiti Putra Malaysia. The plants were prepared using the method describes previously by Sripandikulchai et al.,5 and Zakaria et al.,6 with slight modification. Briefly, the freshly collected roots were washed and weighed. After that the parts were cut in small and dried at 50 to 60°C for 5 days. The dried parts were weighed and then ground to obtain the powder form of plants. The dried powders of plants were soaked in 2 liter of distilled water for 2 hours at room temperature. Then, the mixture boiled until the volume was decreased to one-third of the initial volume. After boiling, the mixture was cooled and kept at 60 to 80°C for an hour. The mixture was filtered using Whatman filter paper No.1 and the filtrate collected. The filtrate was then freezing dried and final
extract was stored at minus 20°C prior to use (yield: C. papaya 14.8% w/w and A. comosus 16% w/w). The extracts were reconstituted with distilled water prior to diuretic assessment in different concentration of the treatment.

**Animals and Experimental Design**

Twenty-one adult male Spraque-Dawley rats (180-200 g) were housed in cages of three. They were first acclimatized to the laboratory environment for 7 days prior to experiment. They were maintained under standard and uniform laboratory condition with free access to rat pellet diet and water *ad libitum*. All animals were fasted overnight prior to experiment. All experiments are approved by the University Animal care and use committee (ACUC).

**Diuretic assessment**

Method of Sripanidkulchai *et al.*, and Adam *et al.*, were used for the determination of diuresis. Extracts and drugs were administered orally. Animals were given bicarbonate saline solution (40 ml/kg) 30 minutes before the experiment. They were divided into 7 groups and placed in an individual metabolic cage. Negative control (Group 1) was given 1 ml distilled water orally. The positive control groups (Group 2 and 3) were given furosemide and hydrochlorothiazide (Sigma Chemicals, US) at 10 mg/kg in distilled water respectively. Treatment groups (Groups 4-7) were given plants extracts at a dose of 5 and 10 mg/kg for each rat. Animals were then put into metabolic cages individually. Water and feed were available to the animals. Urine sample were collected and measured hourly for 4 hours. Urine density and urine pH were also measured hourly. The content of urinary electrolytes that included sodium (Na⁺), potassium (K⁺) and chloride (Cl⁻) was determined by using ion selective electrodes (ISE) automatic analyzer (Radiometer EML 100, Copenhagen).

**Blood serum analysis**

Rats were anesthetized using pentobarbitone (50 mg/kg ip) and intracardiac blood samples (3 to 5 ml) were taken. Rats eventually sacrificed by cervical dislocation. Blood samples were centrifuged at 4000 rpm for 10 minutes. The serum was separated and frozen at -20°C for analysis of glucose, albumin, blood urea nitrogen (BUN) and creatinine level. These tests were performed with an automatic chemical analyzer (Hitachi Roche 902, Japan).

**Statistical Analysis**

The results are presented as mean ± standard error of mean (S.E.M). All assessment data were analyzed using One-way analysis of variance (ANOVA) and result will be considered significant if (p<0.05). Post hoc test that used is Duncan Test that can be determined significant statistically difference between the group of experiment.

**RESULTS AND DISCUSSION**

**Urine Volume**

Table 1 shows volume of all treatment groups. The extract of *C. papaya* (10 mg/kg) produced maximum excreted volume which was similar to that of positive control, furosemide and hydrochlorothiazide. On the other hand, the extract from *A. comosus* showed less potent diuretic action.

**Urinary electrolytes**

The amount of urinary sodium, potassium and chloride were measured every hour from the collected urine, as shown in Table 2. The positive control groups; furosemide and hydrochlorothiazide, excretion of sodium and chloride were markedly increased every hour throughout the experiment compared to negative control. *C. papaya* (10 mg/kg) and *A. comosus* (5mg/kg) were slightly increased urinary sodium excretion compared to the negative control. In the other hand, *A. comosus* (10mg/kg) had the similar pattern of sodium excretion to the negative control group. The extract from *C. papaya* (5 mg/kg) produced reduced amount of the urine sodium compared to the negative control.

Both plant extracts showed markedly increased amount of the urinary potassium every hour throughout the experiment compared to the negative control. Total urinary potassium for both plants aqueous extracts are significant compared to the negative control. *C. papaya* aqueous extracts revealed dose-dependently effects; however, *A. comosus* activity was dose-independently, lower dose (5 mg/kg) of *A. comosus* more potent compared to the high dose (10 mg/kg). Among the two plant extracts tested, a higher amount of the urinary potassium excretion was observed in the group that treated with *A. comosus* at dose 5 mg/kg during the third hour.

Both plant extracts showed moderate increased amount of the urine chloride every hour throughout the experiment compared to the negative control; but the difference was not statistically significant. The urinary chloride excretion profiles of both plant extracts were similar.

**Blood serum analysis**

The effects on glucose, albumin, blood urea nitrogen (BUN) and creatinine concentration in rat’s blood serum treated with aqueous extracts of plants are summarized in Table 3. The concentration of blood serum glucose, albumin, BUN and creatinine were measured after the diuretic assessment. The result showed that both plants extract slightly increased the concentration of glucose. *C. papaya* extract at dose 5 mg/kg revealed decreased glucose concentration. However, the change was not statistically significant relative to negative control.

Both plant extracts were markedly increased the albumin, BUN and creatinine concentration, statistically significant relative to negative control. The action of *A. comosus* was...
dose-independent to albumin and creatinine concentration. Otherwise was dose-dependent to BUN concentration. C. papaya was dose-independents to albumin, BUN and creatinine concentration.

This study revealed that the aqueous extracts from the roots of C. papaya and A. comosus have a demonstrable diuretic activity. Furosemide caused the expected increase in the urine volume, renal excretion of Na⁺ and K⁺, whereas had relatively no effect on Cl⁻ loss. Furosemide acts by inhibiting electrolyte reabsorption in the thick, ascending limb of the loop of Henle\(^1\,^1\,^2\). On the other hand, hydrochlorothiazide enhanced urine output, Na⁺ and Cl⁻ loss significantly. Hydrochlorothiazide inhibits sodium and chloride reabsorption in the distal tubules\(^3\,^7\,^1^1\). The diuretic actions of these both extracts were less potent than that of positive control; furosemide and hydrochlorothiazide, except to C. papaya extract at dose 10 mg/kg. C. papaya extract at dose 5 mg/kg caused a significant increase of urinary output. The increment of urine volume is qualitatively similar potency to hydrochlorothiazide and furosemide\(^1^4\) and the elevated of potassium ions is more potent than the positive control. The effect of diuretic activity of C. papaya extract was dose-independent, whereas A. comosus extract is dose-dependent.

### Table 1: Effects of plant extracts on urine volume

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Urine volume (4 hr in ml/kg body weight)</th>
<th>Diuretics action(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (control)</td>
<td>-</td>
<td>1.073 ± 0.54(^a)</td>
<td>1</td>
</tr>
<tr>
<td>Forusemide</td>
<td>10</td>
<td>25.071 ± 4.79(^b)</td>
<td>23.43</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>10</td>
<td>23.163 ± 0.61(^c)</td>
<td>21.64</td>
</tr>
<tr>
<td>C. papaya</td>
<td>5</td>
<td>11.718 ± 2.17(^b)</td>
<td>10.95</td>
</tr>
<tr>
<td>A. comosus</td>
<td>10</td>
<td>12.092 ± 3.99(^b)</td>
<td>11.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M; \(^1\) Diuretics action = (urinary excretion of treated group (4 hr)) / (Urinary excretion of control group (4 h))

### Table 2: Total excretions over a period of 4 hour of urinary electrolytes

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Na⁺ (mol)</th>
<th>K⁺ (mol)</th>
<th>Cl⁻ (mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (control)</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Forusemide</td>
<td>10</td>
<td>139.67 ± 11.9</td>
<td>140 ± 3.21</td>
<td>117.33 ± 2.73</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>10</td>
<td>159 ± 28.36</td>
<td>227.73 ± 43.92</td>
<td>175.67 ± 24.88</td>
</tr>
<tr>
<td>C. papaya</td>
<td>5</td>
<td>13 ± 1.63</td>
<td>6 ± 0.82</td>
<td>9.33 ± 3.38</td>
</tr>
<tr>
<td>C. papaya</td>
<td>10</td>
<td>30.6 ± 11.2</td>
<td>37.67 ± 0.817</td>
<td>31.7 ± 3.27</td>
</tr>
<tr>
<td>A. comosus</td>
<td>5</td>
<td>37 ± 0.21</td>
<td>28 ± 0.41</td>
<td>25.0 ± 0.1</td>
</tr>
<tr>
<td>A. comosus</td>
<td>10</td>
<td>23 ± 0.13</td>
<td>16.5 ± 4.94</td>
<td>17.33 ± 9.35</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M: ND-Not determined (No urine)

### Table 3: Mean concentration of glucose, albumin, Blood Urea Nitrogen (BUN) and creatinine of rat’s blood serum.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Mean Glucose Conc. (mmol/L)</th>
<th>Mean Albumin Conc. (g/dL)</th>
<th>Mean BUN Conc. (mg/dL)</th>
<th>Mean Creatinine Conc. (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water (control)</td>
<td>-</td>
<td>5.26 ± 0.524</td>
<td>24.0 ± 3.279(^a)</td>
<td>3.86 ± 0.374(^b)</td>
<td>0.59 ± 0.079(^a)</td>
</tr>
<tr>
<td>Forusemide</td>
<td>10</td>
<td>5.78 ± 0.246</td>
<td>28.43 ± 2.563(^a)</td>
<td>5.08 ± 0.421(^b)</td>
<td>0.748 ± 0.059(^b)</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>10</td>
<td>4.13 ± 0.722</td>
<td>16.1 ± 2.933(^b)</td>
<td>3.18 ± 0.709(^b)</td>
<td>0.43 ± 0.090(^b)</td>
</tr>
<tr>
<td>C. papaya</td>
<td>5</td>
<td>4.32 ± 0.233</td>
<td>34.23 ± 1.047(^b)</td>
<td>8.87 ± 0.524(^b)</td>
<td>0.842 ± 0.031(^b)</td>
</tr>
<tr>
<td>C. papaya</td>
<td>10</td>
<td>6.42 ± 0.826</td>
<td>32.72 ± 2.299(^b)</td>
<td>5.8 ± 0.277(^b)</td>
<td>0.792 ± 0.041(^b)</td>
</tr>
<tr>
<td>A. comosus</td>
<td>5</td>
<td>6.03 ± 0.850</td>
<td>35.28 ± 1.164(^b)</td>
<td>11.65 ± 0.542(^b)</td>
<td>0.87 ± 0.052(^b)</td>
</tr>
<tr>
<td>A. comosus</td>
<td>10</td>
<td>5.23 ± 0.067</td>
<td>29.23 ± 0.983(^b)</td>
<td>21.67 ± 0.261(^b)</td>
<td>0.767 ± 0.015(^b)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M; \(^a\) mean with different superscripts differ significantly at (P< 0.05); All assessment data were analyzed using One-way analysis of variance (ANOVA) and Duncan Test is used to determine differences between the treatment means.
The mechanism of action by which diuresis was induced by the plant extracts was also investigated by comparing with standard reference drugs, furosemide (a high-ceiling loop diuretic) and Hydrochlorothiazide (a carbonic anhydrase inhibitor)\(^\text{12,19}\). In general, diuretics act as by increasing the quantity of the sodium ion in the urine, the increased salt excretion being accompanied by an increased water excretion to maintain osmotic balance. Diuretics actions through three ways; by inhibit the reabsorption of sodium ions by a direct action on the kidney tubules, inhibit tubular reabsorption of sodium ions by an indirect mechanism, and increase the filtered load of sodium ions in the glomeruli. In other words, diuretics will give net effect to increase urine volume due to sodium and water excretion\(^1\). The observed decrease of urine density could be explained by a marked increase in urinary flow, which was more important than the urinary electrolytes excretion. The pH alteration that presented involves an accounting of the carbonic (HCO\(_3\)). The carbonic acid system is the most important buffering system of the plasma.

Hydrochlorothiazide blocks Cl\(^-\) reabsorption, creating intraluminal negative charge that impedes Na\(^+\) reabsorption resulting in increased K\(^+\) losses because of increase Na\(^+\) delivery with increased aldosterone. Basically, Hydrochlorothiazide inhibits sodium and chloride reabsorption in the kidney distal tubules and produces a corresponding increase in sodium, water and potassium excretion. Hydrochlorothiazide produces alkaline urine containing increased Na\(^+\), K\(^+\), HCO\(_3\) and Cl\(^-\). Furosemide acts by inhibition of the sodium pump in all part of the nephron and to a lesser extent by inhibition of carbonic anhydrase. They promote the excretion of a higher percentage of filtered salt. Urine that excreted is alkaline and contains excess Na\(^+\), K\(^+\) and HCO\(_3\). It is possible that C. papaya and A. comosus extracts exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying anions, as such action has been hypothesized for some plants\(^\text{14,17,18}\).

Mechanism that presented by C. papaya and A. comosus extracts maybe similar. Our result presented C. papaya and A. comosus extracts significantly increased (P<0.05) potassium ions. C. papaya and A. comosus extracts that studied do not give significant alteration of urinary levels of sodium. Mechanism of action of extracts is not totally analogous to hydrochlorothiazide and furosemide. The diuretic action observed may depend on stimulation of the urinary tract and is linked to the activation of neurohumoral mechanism, mediators of stimuli acting on glomerules, tone acid on the pyelo-uretral peristaltis\(^\text{19}\). The diuretic activity of C. papaya may associate with the high salt or electrolyte content of its extract. These effects might be due to the influence that the electrolytes, present in considerable quantities on the plant, exert on renal epithelium\(^\text{19}\). However, this activity appeared to not correlate well with the maximum urine volume and the amount of electrolytes excreted during the first hour of urine collection\(^\text{5,14}\).

The plant extracts exerted its diuretic activity by inhibiting tubular reabsorption of water and accompanying anions\(^\text{17,18}\). The other possibility for the observed diuretic properties could be due to direct action of K\(^+\) content of C. papaya extracts caused by highest potassium ions contents\(^\text{14,20}\). Our results indicated that the existing diuretic activity of both plants seems thus to be mediated through a change in potassium transport. Plant extracts may be inhibiting potassium absorption or stimulating potassium secretion, or both, leading, in either case, to more potassium retention in the lumen of the kidney tubules and osmotic water flow\(^\text{21}\).

The mechanism of action of the plant extracts on the urine and electrolyte output may not totally be identical. This could be due to the different active components (or mechanisms) extracted. The data presented in this study indicate that plant extracts contained compound(s) that mediated diuretic effects by increasing the rate of urine output as well as electrolyte excretion\(^1\).

In the present study, the diuretic effect observed does not exclude the possibility that changes in the diuresis may occur as a consequence of the presence of polar drug or active compounds\(^\text{22}\), for examples flavonoid glycosides\(^\text{23-25}\), saponosids\(^\text{25}\) and ascorbic acid\(^\text{26,27}\). These natural compounds might be acting synergetically or individually promoting an initial vasodilatation\(^\text{28}\). It is also possible that plant extracts might manifest cumulative effect of several substances in the extract and/or due to secondary active metabolite\(^\text{29}\). Other monovalent and bivalent cations are present in this plant and might have a diuretic activity synergetically with K\(^+\)\(^\text{19,30}\).

Results from the present study demonstrate variation changes of concentration of the parameter in the blood serum analysis. There are significant change occurred in the blood chemistry parameters, including creatinine, blood urea nitrogen (BUN), and albumin of treated group. Glucose level was slightly increases but not significantly for both plants extracts. Even though, this finding is not enough to categorize as diabetic. The mechanism of action of the extract is unknown in this case due to the increasing of glucose level is not correlated well with dose of extract.

**CONCLUSION**

As a conclusion, A. comosus, and C. papaya plant extracts exhibited diuretic properties. The finding results show that A. comosus and C. papaya are paralleled and totally agree with the ethnomedical information for those plants that used for dysuria treatment. However, both extracts also increased serum level of albumin, BUN and creatinine indicating potential nephrotoxic effects. Therefore, care should be taken when patients ingesting these herbs.

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REFERENCES


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