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The in vivo antihypertensive effects of standardized methanol extracts of Orthosiphon stamineus on spontaneously hypertensive rats: A preliminary study

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Outline

• Introduction
• Objective
• Hypothesis
• Methodology
• Results
• Discussion
• Conclusion
Introduction

• Hypertension causes 4.5 % of current global disease burden.

  The World Health Organization, 2003

• Prevalence: ~ 4.8 million individuals in Malaysia - aged 30 years old and above.

  Norzila et al, 2009

• HPT is a risk factor for cardiovascular, cerebrovascular and renal diseases

  The World Health Organization, 2003
## Synthetic anti-HPT

<table>
<thead>
<tr>
<th>DRUG</th>
<th>COST PER DAY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Thiazide diuretics</strong></td>
<td>About 7 cents</td>
</tr>
<tr>
<td>Cause kidneys to increase urine, removing excess fluid from blood vessels. Diuretics, or water pills, have minimal side effects.</td>
<td></td>
</tr>
<tr>
<td><strong>Beta blockers</strong></td>
<td>A dime to a dollar</td>
</tr>
<tr>
<td>Make the heart beat slower by blocking nerve-stimulating hormones. They've been used for 40 years for hypertension, angina, migraines and anxiety.</td>
<td></td>
</tr>
<tr>
<td><strong>ACE inhibitors</strong></td>
<td>About $1</td>
</tr>
<tr>
<td>Expand blood vessels by blocking certain hormones. ACE inhibitors are linked to substantially more strokes, compared to diuretics.</td>
<td></td>
</tr>
<tr>
<td><strong>Angiotension II antagonists</strong></td>
<td>About $1</td>
</tr>
<tr>
<td>Work like ACE inhibitors, but with fewer major side-effects. These are the newest class of antihypertensive drugs.</td>
<td></td>
</tr>
<tr>
<td><strong>Alpha blockers</strong></td>
<td>More than $1</td>
</tr>
<tr>
<td>Slow the brain’s action on the nervous system, lowering blood pressure.</td>
<td></td>
</tr>
<tr>
<td><strong>Calcium channel blockers</strong></td>
<td>About $2</td>
</tr>
<tr>
<td>Relax blood vessels by interrupting the movement of calcium into nearby muscles. While as effective as diuretics, calcium blockers are linked with 25 to 38 percent more heart attacks and increased rates of depression and suicide.</td>
<td></td>
</tr>
</tbody>
</table>

*ALDO CHAN / THE SEATTLE TIMES*

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The cost management is expensive
Undesirable side effects

Seok-Koo et al, 2009
Norzila et al, 2009
Table 82. Barriers Associated with Nonadherence to Blood Pressure Medications

- Inadequate insurance coverage for medications and medical treatment
- Lack of understanding
- Sexual side-effects
- Cultural issues related to dependence on medication
- Poor communication between patient and physician
- Drug intolerance
- Less than optimal interface between primary and specialty care for patient
- Literacy
- Hearing impairment
- Cognitive impairment
- Lack of resources for care
- Patient’s belief system
- Inadequate support system
- Lack of motivation to adhere to treatment plan
- Perception of decreased quality of life while taking antihypertensives
People nowadays are more interested in the usage of dietary herbs and medicinal plants as an alternative management for hypertension. This is because of its minor side effects and has well therapeutically performance.

Maghrani et al, 2005
In Malaysia, *Orthosiphon stamineus* (OS) is believed to have antihypertensive properties.
Objective

- To investigate the effect of two weeks oral OS on the SBP of spontaneously hypertensive rats (SHR).
Hypothesis

- **Null**: There is no significant mean reduction of SBP after 2 weeks OS administration
- **Alternative**: There is a significant mean reduction of SBP after 2 weeks OS administration
Methods and Materials

• Randomized Control Trial
• Laboratory Animal Research Unit (LARU), USM
• Approved by Animal Ethic Committee of Universiti Sains Malaysia in December 2010

(Ref USM/ Animal Ethics Approval/2010/ (63) (257)
Plant material and extraction

• About 265 g of standardized methanol extracts of OS was provided by Prof. Zaini Asmawi from School of Pharmaceutical Sciences, USM.

[OS leaves were extracted with 50 % methanol using a maceration method (200 g of dried leaves in 2 l of 50% ethanol at 55°C for 24 hours, 2 cycles). The extract was then concentrated and freeze-dried, resulting a yield of dried leaf]

Mohamed et al, 2011
Selection of animals

• Adult male SHR (12 weeks old)
• Weight: 150-250g.
• A week of acclimatization
• The handling and use of animals were in accordance with the institutional guidelines
15 SHR randomly divided into 5 groups

<table>
<thead>
<tr>
<th></th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>20 mg/kg Irbesartan</td>
<td>Distilled water</td>
<td>250 mg/kg</td>
<td>500 mg/kg</td>
<td>1000 mg/kg</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

**DAY 0**

SBP was measured via blood pressure detector (Norazmir et al, 2010)

**DAY 1 to DAY 14 (2 WEEKS)**

Irbesartan/distilled water/OS was administered orally (10 ml/kg) via oral gavage once a day (Maghrani et al, 2005)

**DAY 14**

SBP was measured after treatment via blood pressure detector (Norazmir et al, 2010)

**DAY 15**

animals were sacrificed with CO₂

**STATISTICAL ANALYSIS**

means ± SD + Student t-test

**ANALYZE DATA**
Taking BP of the rats is the MOST DIFFICULT PART!!!
Statistical analysis

- Statistical Package for Social Sciences (SPSS) 12.0 software for Windows.
- Results were expressed as mean and SD of three observations in each group.
- Student’s t-test was used to compare the means (pre and post intervention).
- Differences were considered statistically significant at \( p < 0.05 \).
Results

*All groups achieved mean SBP < 120 mmHg except –ve control group*
*The minimum dose of OS to achieve control SBP was 250 mg/kg*
Table 2. The significance of antihypertensive effect of OS among SHR (Student’s T-test).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean of SBP difference (95% CI*)</th>
<th>t-statistic (df)</th>
<th>**P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>42.33 (1.95, 82.72)</td>
<td>4.51 (2)</td>
<td>0.046</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.00 (-6.57, 6.57)</td>
<td>0.00 (2)</td>
<td>1.000</td>
</tr>
<tr>
<td>Group 3</td>
<td>41.00 (21.13, 60.87)</td>
<td>8.88 (2)</td>
<td>0.012</td>
</tr>
<tr>
<td>Group 4</td>
<td>26.67 (17.94, 35.39)</td>
<td>13.15 (2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Group 5</td>
<td>37.67 (2.42, 72.91)</td>
<td>4.60 (2)</td>
<td>0.044</td>
</tr>
</tbody>
</table>

*CI: confidence interval; **P-value is less than 0.05 in groups 1, 3, 4 and 5.
Summary

• OS at 250, 500 and 1000 mg/kg have significant antihypertensive effect
• OS at above 250 mg/kg does not further reduce the SBP
• OS at 500 mg/kg has better statistical and clinical significance
• The efficacious of OS as antihypertensive is comparable with irbesartan 20 mg/kg
The possible mechanism

- Os appears to influence the activity of adenosine A\(\text{(1)}\) receptor antagonists to increase the volume of urinary excretion (diuresis)

Arafat et al, 2008
Yuliana et al 2009
Adam et al, 2009
Recommendations

• Continuation of similar study with a calculation of sample size

• Two different species of animals to compare any changes of SBP level between normotensive and hypertensive rats.

• Two different methods of blood pressure measurement – i.e.; direct (tail cuff method) and indirect measurement (cannulation).
Conclusion

• In vivo, OS has antihypertensive potential in SHR and its efficacious is comparable to modern recent anti-HPT agent.

• Future Human Clinical Trial is needed to approve its anti-HPT benefit
Acknowledgement

• Advanced Medical and Dental Institute of USM
• School of Pharmaceuticals Sciences, USM Penang
• Hospital Universiti Sains Malaysia
• Department of Physiology
• Laboratory Animal Research of USM Health Campus
Darah tinggi kepala pusing
Kepala pusing, sakit dada
Usah risau, usah rungsing
Misai Kucing kan ada

THANK YOU