THE ANTI-HYPERTENSIVE EFFECTIVENESS OF CONNARUS GRANDIS JACK. LEAVES EXTRACT ON DIFFERENT MODELS OF HYPERTENSIVE RATS

Armenia¹, Welmidayani¹, Rusdi¹ and Munavvar²
¹Jurusan Farmasi FMIPA Univ. Of Andalas, Padang, Indonesia
²School of Pharmacy, University Science Malaysia, Penang Malaysia

Abstract

The effectiveness of Connarus Grandis Jack. leaves ethanolic extract on different models of hypertensive rats has been observed. Anesthetized spontaneously hypertensive rats (SHR) and 2K1C-Goldblatt hypertensive rats were treated with Connarus Grandis Jack. extract at doses of 10, 20, 40 and 80 mg/kg every half an hour for 1½ hours. Groups of saline and Captopril treated rats were used as a control and a comparison respectively. Direct systolic, diastolic, and mean arterial pressure and the heart rate were recorded for ten minutes before each doses. Results showed that all parameters were reduced significantly in both hypertensive models (p<0.05), except heart rates, in 2K1C rats did not change significantly (p>0.1). The percentage reduced in blood pressures were higher in SHR as compared to in 2K1C rats. These indicated that the effectiveness of Connarus Grandis leaves extract is better in essential than renovascular hypertension.

Keywords: Connarus Grandis Jack, hypertension, SHR, 2K1C-Goldblatt rats.

Introduction

Traditional medicines has a very long history: it is the sum total of the practices based on the theories, beliefs and experiences of different cultures and times, often inexplicable, used in the maintenance of health, as like in the prevention, diagnosis, improvement and treatment of illnesses. In every country traditional medicines find foundation in magical or religious beliefs, or popular experience and the World Health Organization is engaged to establish definitive guidelines for methodology of clinical research and the appraisal of effectiveness of traditional medicine (Firenzuoli and Gori, 2007).

Herbs are staging a comeback and herbal ‘renaissance’ is happening all over the globe. The herbal products today symbolise safety in contrast to the synthetics that are regarded as unsafe to human and environment. Although herbs had been priced for their
medicinal, flavouring and aromatic qualities for centuries, the synthetic products of the modern age surpassed their importance, for a while. However, the blind dependence on synthetics is over and people are returning to the naturals with hope of safety and security (Joy et al., 1998).

The World Health Organization estimates that approximately 80 percent of the world’s population relies primarily on traditional medicines as sources for their primary health care (Farnworth, et al., 1985). The reason is that a complex pathogenesis of many diseases makes a mono-substance therapy dogma change to multi-substances therapy to obtain multi-target therapy. This means, that a combination drug that should be prescribed by a physician could be replaced by multi-component content of an herbal.

Hypertensive takes long time or even long-life therapy since the disease is diagnosed. Most of anti-hypertensive drug is expensive or unsaved if used for such long time. *Conarus grandis* Jack. Connaraceae is one of medicinal plant originated from Indonesia and has been documented at the Kebun Raya, Bogor. It is a woody climbing tree and found in Sumatran, Jawa and Bangka (Backer & Backhuizen, 1965). According Heyne (1950), the bark of this plant is used for asthma, while Lewis (1977) described that, different of Connaraceae family used for oral infection, and according to Burkil (1966), some Connarus genus are used to treat worm infection, fever, asthma and itching. Armenia (1990) found that the ethanolic extract of *Conarus grandis* leaves active as central nervous system depressant, sympatholytic/ parasympathomymetic, muscle relaxant and or vasodilator. These facts were approved by a prolong sleeping time of mice induced by pentobarbitone (Armenia, 1990; Sulastri, 1993), dan Yuniar (1997), retracted chicken head (muscle relaxation)(Armenia dan Arifin, 1992), and antagonizes
convulsion induced by strychnine nitrate (Armenia, 1995) and has anti-bacterial property (Armenia dan Akmal, 1996). The most important is that the leaves decreased BP of normotention rats (Armenia, 1990, Noveri et al., 1994, Permana-Sari et al., 1997), and for most of all, the crude ethanolic extract posses a better activity as compared to fractions or isolated compound. So far there is no further information regarding the antihypertensive effectiveness of the plant on hypertensive animal models.

The objective of this research is to study the effectiveness Connarus grandis Jack. Leaves ethanolic extract to lower the BP on different model of hypertensive rats, i.e. spontaneously hypertensive rats (SHR) and the two kidney one clip (2K1C) Goldblatt hypertensive rats.

**Material and Methods**

*Extract preparation*

An amount of 3 kg of Connarus grandis fresh leaves taken from Kebun Raya Bogor, was air dried at room temperature (29°C) until 1.4 kg of weight and then macerated with 70% ethanol for 3x5 consecutive days. The extract then evaporated in-vacuo and then air dried until 44.72 g dried extract was obtained (10.69 of moisture and 3.26% of ash content).

*Preparation 2K1C-Goldblatt hypertensive rats*

The 2K1C-Goldblatt hypertensive rats were prepared by a method described by Armenia (2001), Vogel, 2002,) and Badyal et al., (2003) with modification (left the renal artery was tied with thread instead of clipping with silver clip to narrow (0.2mm width) the artery. The rats with SBP of > 150 mmHg after 1 month clipping were used in this experiment.
Anti-Hypertensive Assay.

Two series of experiments were conducted, Seri 1 on SHR and the 2nd Seri on 2K1C-Goldblat hypertensive rats. Each Seri consist of 6 groups (3 rats each), Group 1 of each Seri were as controls (treated with saline), Group 2, 3, 3 and 4 were treated with Connarus grandis leaves extract at doses of 5, 10, 20 and 40 mg/kg respectively and the last group rat was treated with Captopril (2.5 mg/kg) for comparison. Rats were anesthetized (pentobarbitone sodium, 60 mg/kg i.p.). A plastic canula was inserted into trachea to facilitate breathing, the jugular vein was canulated for infusion of anesthesia and the carotid artery was canulated BP monitoring and finally the rat bladder was canulated to facilitate spontaneous urine flow. The direct blood pressure (systole =SBP), diastole (DBP) and mean arterial pressure (MAP) and the heart rate were monitored and recorded (MP 150 Biopac System) before and every half an hour after dosing (3 repeated doses). Note: During experiment, rats were infused with saline containing pentobarbiton (2.5 mg/kg/h) sodium 2 ml/minutes to maintain anesthesia. Data were analyzed using the Two Way ANOVA (SPSS) followed by Duncan Multi-factorial T-Test. The significance was taken at p< 0.05.

Results

BP (MAP, SBP, DBP) and HR in SHR were highly significant decreased by Connarus grandis extract (p<0.0001) but not in 2K1C (p>0.1), while repetitive doses did not significantly influence all parameter in both kind of rats (p>0.1), but interaction of dose-repetitive dose mostly tent to influence these parameters (p<0.1) (Figure 1 to 8).
In this situation, the decrease of MAP in SHR treated with CG extract according to doses with averages 7 – 20%. Repetitive lower doses of CG extract increased the % decrease of MAP but at higher CG extract dose tent to decrease the MAP. The higher % decrease of SHR MAP treated with CG extract was not significantly different as compared to those in captopril treated rats (p>0.1)(Figure 1 left, Table 1). On the other side, CG extract was not significantly MAP of 2K1C rats as compared to control (p>0.1) and rats treated with Captopril (p>0.1), but the decrease of MAP in 2K1C rats treated with Captopril was significantly different as compared to control rats (p<0.05). Further more there is no influence of repetitive doses of CG extract nor its interaction with doses to the MAP of 2K1C rats (p>0.1)(Figure 1 right Table 2)

Figure 1. Effect of *Connarus grandis* extract (Seri 2-5) and Captopril (Seri 6) with 3x doses repetition to MAP of SHR (left) and 2K1C rats (right).
SBP of SHRs were significantly (p<0.0001) decreased by the doses CG extract, interaction of doses-repetitive dose (p<0.05), but not by repetitive doses (p>0.1). The % decrease of SBP in SHR treated by CG extract of different doses were from 7 – 21% (Tabel 1). Similar with MAP, the % decreased of MAP were increased when the doses were repeat, but at higher dose, repetitive decreased the % decreased of SBP. The higher % decrease of SBP on CG extract treated SHR was not significantly (p>0.1) with those rats treated with Captopril 2.2 mg/kg (p>0.1)(Figure 2 left,Table 1). In 2K1C rats, the influence of CG extract to SBP was not significant (p>0.1) nore repetitive doses and its interaction with dose (p>0.1), even though SBP of 2K1C rats treated with CG extract at lower dose decreased by ± 4%, it was not significantly different with those rats treated with Captopril, which decrease the SBP significantly as compared to control (Figure 2 right, Table 2).

Figure 2. Effect of *Connarus grandis* extract (Ser 2-4) and Captopril (Seri 6) with 3x doses repetition to SBP of SHR (left) and 2K1C rats (right).

DBP
The influence of CG extract to DBP of SHR was similar to that of MAP and SBP (p<0.0001). DBP of SHR treated with CG extract decreased from 9 – 21% according to doses. Repetitive lower doses increased the % decrease of DBP, but at higher dose the % decrease of DBP was lower. The highest % decrease of DBP of CG extract treated rats was not significantly different with % decrease of DBP of Captopril treated rats (p>0.1). (Figure 3 left, Table 1). On the other hand, the DBP of 2K1C rats was not significantly influence (p>0.1) by CG extract doses, tent to influence by repetitive dose (p<0.1), but not by interaction of doses-repetitive doses (p>0.1). The DBP of 2K1C rats treated with CG extract relatively lower but not significant as compared to control rats while the DBP of rats with Captopril decreased by ± 8%. The % decrease of DBP rats treated with repetitive dose of CG extract was irregular (Figure 3 right, Table 2).

Figure 3. Effect of *Connarus grandis* extract (Seri 2-4) and Captopril (Seri 6) with 3x doses repetition to DBP of SHR (left) and 2K1C rats (right).

**Heart Rates (HR)**
Heart rate of SHR treated by *Connarus grandis* extract was significantly (p<0.001) decreased as compared to control rats, but repetitive doses did not influence this parameter nor as doses-repetitive doses interaction (p>0.1). The decrease of HR in these rats at higher dose was not significantly different as compare to that on Captopril treated rats (p>0.1). In 2K1C rats, neither dose, repetitive doses nor their interaction affect the HR significantly (p>0.1), while Captopril decreased SHR HR significantly (p<0.01) of 3.2% compared to that in control rats of 0.4% (Figure 4, Table 1 & Table 2).

![Graphs showing effect of Connarus grandis extract and Captopril on HR of SHR and 2K1C rats](image)

**Figure 4.** Effect of *Connarus grandis* extract (Seri 2-4) and Captopril (Seri 6) with 3x doses repetition to HR of SHR (left) and 2K1C rats (right)

**Table 1.** The effect of *Connarus grandis* leaves extract to the percentage reduced in BP on SHR

<table>
<thead>
<tr>
<th>No.</th>
<th>Doses (mg/kg)</th>
<th>Percentage reduced of BP and HR</th>
<th>MAP</th>
<th>SBP</th>
<th>DBP</th>
<th>HR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MAP</td>
<td>SBP</td>
<td>DBP</td>
<td>HR</td>
</tr>
<tr>
<td>1</td>
<td>K</td>
<td></td>
<td>0.30 ± 0.43 a</td>
<td>0.30 ± 0.43 a</td>
<td>0.40 ± 0.62 a</td>
<td>0.27 ± 0.32 a</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td></td>
<td>7.43 ± 0.91 b</td>
<td>7.58 ± 0.98 a</td>
<td>9.36 ± 0.59 b</td>
<td>4.92 ± 0.46 b</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td></td>
<td>14.29 ± 2.92 c</td>
<td>16.62 ± 3.82 bc</td>
<td>11.29 ± 1.91 b</td>
<td>9.75 ± 1.09 b</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td></td>
<td>16.15 ± 2.93 cd</td>
<td>17.35 ± 3.95 c</td>
<td>21.17 ± 3.33 c</td>
<td>18.30 ± 2.87 c</td>
</tr>
<tr>
<td>5</td>
<td>80</td>
<td></td>
<td>20.15 ± 2.35 d</td>
<td>20.75 ± 2.72 c</td>
<td>19.73 ± 2.14 c</td>
<td>17.12 ± 2.63 c</td>
</tr>
<tr>
<td>6</td>
<td>Captopril</td>
<td></td>
<td>18.60 ± 2.34 cd</td>
<td>17.6 ± 2.95 c</td>
<td>19.30 ± 1.27 c</td>
<td>16.41 ± 1.98 c</td>
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Table 2. The effect of *Connarus grandis* leaves extract to the percentage reduced in BP on 2K1C-Goldblatt hypertensive rats.

<table>
<thead>
<tr>
<th>Kel.</th>
<th>Dosis (Mg/kg)</th>
<th>Percentage changed of BP and HR</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TDS</td>
<td>TDD</td>
<td>MAP</td>
<td>HR</td>
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<tr>
<td>1</td>
<td>K</td>
<td>0.6522&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7644&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2111&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.0211&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>-4.1711&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-1.9289&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-3.2689&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-.8300&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
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<td>-2.9878&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>-3.4800&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>5</td>
<td>80</td>
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<td>-2.4489&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Captopril (2.5)</td>
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<td>-8.4111&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-8.1567&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-6.157&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
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<td>1.21</td>
<td>2.09</td>
<td>1.69</td>
<td>2.39</td>
<td></td>
</tr>
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</table>

Discussion

Normally, the rat BP is similar to those in normal human being, but the heart rate on rat is higher, that is 70 – 80 in human compare to 320 – 480 bpm in rats. The normal systole, diastole and mean arterial pressure is 75 – 120 mmHg, 60 – 90 mmHg, 65 – 100 mmHg respectively.. BP more than tese values will be categorized as hypertension (Badyal, 2003).

Two types hypertension rats were used this anti-hypertension study, such as spontaneously hypertensive rats (SHR) and the renal hypertensive rats (2K1C-Goldblatt hypertensive rats. SHR is a genetically hypertensive rats with MAP normally of 150 – 190 mmHg (Jane et al., 1999 and Armenia, 2001). The renal hypertensive rats is prepared by clipping one or both left and right renal artery (ies) (Badyal, 2003). In this study, the renal hypertensive rats prepared by clipping the left renal artery of (2K1C) tof
normal Wistar-Kyoto rats for one month. With this technique, the MAP of the rats was 117 – 156 mmHg for all (100%) rats, similar as compared to previous study (100 – 150) (Iversen et al., 1998; Armenia, 2001 dan Badyal, 2003). Even though Zeng et al. (1998), reported that only 70% of Sprague Dawley rat with one of the two renal artery was clipped produced hypertension (MAP of 150 mmHg) 3 weeks after clipping.

Doses of 10, 20, 40, dan 80 mg/kgBB used in this study are based on previous study by our team (Armenia, 1990; dan Noveri, 1994) with a little justification. Direct BP monitoring (Newsha, 1998) will give an exact arterial pressure. With Biopac® System MP150 for BP monitor, the MAP, SBP, DBP and HR data can be displayed separately at the same time.

This study provides new evidence that *Connarus grandis* leaves extract produce anti-hypertensive activity. This is in agreement with the previous study on this plant extract (Armenia, 1990; dan Noveri, 1994). Armenia (1990) reported, that ethanolic extract of the plant leaves produced parasympathomymetic and or sympatholytic activities besides muscle relaxant and central nervous systym (CNS) deppressant. Furthermore, Noveri (1994) described that the hypotensif effect of this plant originated from the polar fraction (water), while the non polar (ChCl3) fraction poses a little effect. Blood pressure lowering effect of *Connarus grandis* extract is dose-related and this effect at higher dose was similar as that showed by captopril.

As written in many textbook, a sympatholytic reduce BP via several vays, i.e. by reducing cardiac output doe to its $\beta_1$-adrenoceptor inhibition, vasodilation through $\alpha_1$ or $\beta_2$-adrenoceptor inhibition or by it may block the chatecholamin neurotransmiter release (Tripathi, 2004; Rang, 2006). As a parasympathomymetic, it also produce vasodilation,
or reduce cardiac work and its output by slowing the sinoatrial (SA) nods conduction (Katzung, 2001). The parasympathomymetic activity also may increase the urine flow (as also seen in the toxicity study, data is not included), which in turn decrease the blood volume (Rang et al., 2006). All of these activities will decrease the BP (Sherwood, 2007). The muscle relaxasation activiti of this extract (Armenia, 1992) on the other hand also may explain the hypotensive effect. This effect in aggement with a study reported by Virmani et al., (2006) that Vecuronium and rocuronium (muscle relaxants) decrease the heart rate followed by significant decrease in systolic, diastolic and mean arterial pressures, as seen in this study, especially in SHR.

The most interesting thing in this study is the different effectiveness of *Connarus grandis* leaves extract in these two different models of hypertension. In SHR, this extract seems tobe more effective in reducing blood pressure (MAP, SBP, DBP and HR), while in 2K1C rats was less effective. This means that genetic and pathogenesis of hypertension factors influence *Connarus grandis* extract activity. This situation could be explained through the dynamic of receptor, that may change in density or efficacy, particularly in physiopathologic condition (Armenia, 2001; Tripathi, 2004). This in lead to change the respons of anti-hypertensive drug as seen in this study.

From this study it can concluded that *Connarus grandis* leaves extract effective as anti-hypertension aspecially in genetic hypertension but less effective to renal hypertension.

**References**

Armenia, 1990, Penapisan aktvititas farmakodinamik ekstrak etabol daun akar mambu (*Connarus grandis* Jack, Connaraceae, Thesis S2, ITB, Bandung.)

Armenia, 1995, Isolasi dan skrining antimikroba senyawa aktif dari daun tumbuhan *Connarus grandis* Jack. Laporan Proyek Penelitian OPF.


Armenia, 2001, Neural control of renal haemodynamics, the role of 1-adrenoceptor subtype(s), Disertasi S3, USM Malaysia.


Heyne, K., *De Nullige Planten van Indonesie,* In Twee Delen, Vol. 1, Ser Dreed, C.V., Uitpgeverij Van Hoeve’s, Gravenhage, Bandung, 1950


Noveri, H., Uji Efek Hipotensi Beberapa Fraksi Ekstrak Daun Akar Mambu (Connarus grandis Jack.), Skripsi Sarjana Farmasi Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Andalas, Padang, 1994


