Diuretic Activity and Acute Toxicity of Combination *Eurycoma longifolia* Extract and Irbesartan

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Abstract
"Pasak bumi" (*Eurycoma longifolia* Jack.) has been used traditionally for aphrodisiac, antimalaria, activities as anticancer, antidiabetic, and antihypertension. *E. longifolia* can be used with synthetic hypertensive drugs like irbesartan. This study was to investigate the diuretic effect of *E. longifolia* extract and its combination with irbesartan in normal rats, determinate the LD_{50} value and the toxic effect that influence the organ weight and histopathology of mice. Aqueous extracts of *E. longifolia (*40 mg/kgBB*)* and its combination with irbesartan (20, 40, 80 mg/kgBB) were administered orally to experimental rats strain *Sprague Dawley*. The concentration of sodium and potassium from urine were measured with AAS. The data was analyzed with ANOVA one way. The acute toxicity test was determinated by Weil method. The dose of combination *E. longifolia* extract-irbesartan were 1000 mg, 2000 mg, 4000 mg and 8000 mg/kg. The observation was carried out in 14 day including clinical signs, percentages of death and histopatology of mice organs. The result showed that all of tested group have significant diuretic activity ($p<0.05$) compared with control group, there's no significant difference between single dose extract of *E.longifolia* (40 mg/kgBB) and irbesartan (40 mg/kgBB) but the combination of both have lower activity. The combination of *E. longifolia* extract with irbesartan have LD_{50} value 23.951 g/kg (categorized as non toxic compound). The result of histopathological showed that there're no significant pathologic effects in the kidney, liver and heart.

Keywords: Acute toxicity, diuretic, *E. longifolia*, irbesartan, herb-drug interaction

Aktivitas Diuretik dan Toksisitas Akut Kombinasi Ekstrak *Eurycoma longifolia* dan Irbesartan

Abstrak
Pasak bumi (*Eurycoma longifolia* Jack.) telah digunakan secara tradisional untuk afrodisiak, antimalaria, antikanker, antidiabetes, dan antihipertensi. *E. longifolia* dapat digunakan dengan obat hipertensi seperti irbesartan. Penelitian ini untuk menyelidiki efek diuretik ekstrak *E. longifolia* dan kombinasi dengan irbesartan pada tikus normal, penentuan nilai LD_{50}, efek toksik yang memengaruhi berat badan organ, dan histopatologi tikus. Ekstrak air dari *E. longifolia* (40 mg/kgBB) dan kombinasi dengan irbesartan (20, 40, 80 mg/kgBB) secara oral kepada tikus galur *Sprague Dawley*. Konsentrasi natrium dan kalium dari urin diukur dengan AAS. Data dianalisis dengan ANOVA satu arah. Uji toksisitas akut ditentukan dengan metode *Weil*. Dosis kombinasi ekstrak *E. longifolia*-irbesartan adalah 1000mg, 2000mg, 4000mg dan 8000 mg/kg. Pengamatan selama 14 hari (tanda-tanda klinis, persentase kematian dan histopatologi organ tikus). Hasil penelitian menunjukkan bahwa semua kelompok diuji memiliki aktivitas diuretik signifikan ($p <0,05$) dibandingkan dengan kelompok kontrol, tidak ada perbedaan yang signifikan antara ekstrak tunggal dosis *E. longifolia* (40 mg/kgBB) dan irbesartan (40 mg/kgBB) tetapi kombinasi keduanya memiliki aktivitas yang lebih rendah. Kombinasi ekstrak *E. longifolia*-irbesartan memiliki nilai LD_{50} 23,951 g/kg (senyawa non toksik). Hasil histopatologi menunjukkan bahwa sudah ada efek patologis yang signifikan dalam ginjal, hati dan jantung.

Kata kunci: *E. longifolia*, diuretik, interaksi herbal-obat, irbesartan, toksisitas akut

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Introduction

The hypertension prevalence based on measurement and history of disease was 32.2% with risk factors are geriatric, man, and obesity.[1] There are many conventional antihypertensive which have side effects.[2] The European Society of Cardiology/ Hypertension (ESC/ESH) and JNC (Joint National Committee) VII recommend the diuretic as one of antihypertensive drugs.[3]

The use of herbal as supplement and alternative treatment has been increasing in recent years. People combined herbal and drugs without any consultations with the doctor previously. Many people have the mistaken that being all natural herbs are safe but it is not so. There are many herbs may interact with conventional medicine normally taken simultaneously that obtain serious side reaction. However, there are many herbs have synergist effect for particular drugs.[4]

Eurycoma longifolia Jack have been known by the local names Tongkat Ali in Malaysia and Pasakbumi in Indonesia. It is been used as aphrodisiac, antimalaria, and antidiabetic empirically. Blood pressure can be decreased by intravena injection of *E. longifolia* aqueous extract.[5] The toxicity of *E. longifolia* which is described by LD_{50} value is higher than 5000 mg/kg, there is no significant pathologic on kidneys, liver and testes of rat.[6] *E. longifolia* has many bioactive compounds include the alkaloids and quassinoids form in a major portion (such as eurycomaoside, eurycolactone, eurycomalactone, pasakbumin-B, and eurycomanone).[7]

Both of the effectiveness herbal-drug combination with modern pharmaceuticals and the possible adverse effects from herb–drug interactions remain to be verified. This study was carried out to investigate the diuretic effect of *E. longifolia* extract and its combination with irbesartan in normal rats, also to determinate the LD_{50} value and the toxic effects that influence the organ weight and histopathology.

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Methods

The materials are Male SD rats (175–200 g) and male ddY mice (20–40 g) were purchased from animal house of Bogor Agricultural Institute. They were maintained under standard conditions of temperature and humidity. *E. longifolia* powder was obtained from PT. Deltomed, Solo and Irbesartan was purchased from PT. Indofarma, Bekasi.

E. longifolia powder as much as 800 g was extracted by maceration method then stirring occasionally. Maceration method used aquadest solvent that distilled until a limpid maserat. Aqueous extract (maserat) was concentrated by evaporating solvent using a rotary evaporator at temperature 40 ^{o}C to obtain a thick extract, dried with oven vaccum. Five senses used to describe the organoleptic characteristics of extract observed such as the shape, color, smell, and taste.

The assessment method for diuretic activity based on Sreelakshmi et al.[8] Six groups of six mice each group were fasting and not given water for eighteen hours before the experiment. Rats were placed in metabolic cages for 24 hours, without food or water during this period. The treatment group was given 40 mg/kgBW *E. longifolia* aqueous extract orally, 40 mg/kgBW irbesartan dose and the combination of *E. longifolia* aqueous extract and irbesartan (20, 40 and 80 mg/kgBW). Urine volume is recorded at 2, 4, 6 and 24 hours post administration. Sodium and potassium ion concentrations in urine samples were determined by AAS (Atomic Absorbance Spectrometer).

Male ddY mice were divided into seven groups of six animals each. Control group received a normal saline (2 mL/kg) and the other groups received 1, 2, 4 and 8 g/kg combination of *E. longifolia* extract and irbesartan, respectively. Immediately after dosing, the animals were observed for their behavior continuously four hours for the first. They were kept under observation

up to 14 days after extract administration to find out the mortality, signs toxicity and body weight.

Results

The result of sodium and potassium ion concentrations in urine samples shows that all of groups test increased volume of urine excreted in 24 hours. The concentration of Na^+ and K^+ ion increase significant ($p<0.05$) from control normal group (Table 1).

The combination dose of *E. longifolia* water extract and irbesartan (1, 2, 4 and 8 g/kgBW) caused the mortality of two mice at the highest dose (8 g/kgBW) with 40% mortality response during the 14 hours observation period (Table 2). The mice showed no change in behavior and clinical signs of toxicity such as passive, ataxia, or tremor. LD_{50} values were determined by probit analysis and regression equation was Y=a+bx. Y=-1088+1.39x, where x is the antilog dose (LD_{50})= 23.981 g.

The results of histopatology showed in Table 3. The histology result of liver and heart of acute toxicity looked normal, but there is atrophy of the glomeruli in the kidneys of two mice (8000 mg/kg treatment groups).

Discussion

Diuretics are drugs that capable of increasing levels of urine as well as the electrolyte output, so they are useful in the treatment of diseases that related with the retention of fluids like high blood pressure, or heart failure, and nephrotic syndrome. There are correlations between the volume of urine and the concentration of Na^+, this aspect is logical because the mechanism of action of diuretic drugs is to decrease the tubular reabsorption of this ion, it produces the dragging of the osmotic equivalent of water, other explanation that can support this, is the high ion concentrations in this medicinal plants.

There is no significant difference between single dose of *E. longifolia* extract (dose: 40 mg/kgBW) and irbesartan (dose: 40 mg/kgBW) but the combination of *E. longifolia* extract and irbesartan has lower activity than single extract. So the extract of *E. longifolia* has the same activity with irbesartan, but if its combination the effect of diuretic decrease. It is caused possible by interaction of drug that influences the metabolism. So, the *E. longifolia* extract has same activity with irbesartan, but when combined, the diuretic effect decreases. It is probably caused by drug interactions that affect the metabolism. In addition, irbesartan is one of antihypertensive drugs that has a narrow therapeutic index so that if consumed concomitant with other drugs or herbal therapies may alter the effects of irbesartan. For example irbesartan with garlic or ginseng is able to decrease its effect.[9]

Based on the substance of the table toxicity scale, in practical classification of toxic compounds of the LD_{50} greater than 15 g/kg,[10] so that the combination of *E. longifolia* extracts and irbesartan is safe.

Table 1 Volume of Urine Excreted, Na^+ and K^+ Concentration (Mean ± Standard Deviation, n=6)

No	Group	Volume of urin excreted 24 hours (mL)	Na^+ concentration (mg/dL)	K^+ concentration (mg/dL)
I	Normal (Aquadest)	7.86±1.82	2399	3063
II	Control Na CMC	7.73±1.89	3096	2628
III	Exstract PB, 40 mg/kg	1223±0.75	3386	3620
IV	Irbesartan, 40 mg/kg	12.2±1.21	3441	3154
V	Irb + Extract PB dose 20 mg/BB	10.8±0.95	3986	3813
VI	Irb + Extract PB dose 40 mg/BB	9.97±0.47	3756	3868
VII	Irb + Extract PB dose 80 mg/BB	9.73±1.42	4760	4184

Table 2 Respon of Mortality

Doses (mg/kg)	Number of animal	Mortality of animal	Log doses (x)	Probit (y)
1000	5	0	3	3.36
2000	5	0	3.3	3.36
4000	5	0	3.6	3.36
8000	5	2	3.9	4.75

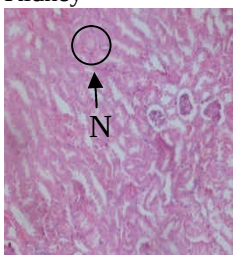
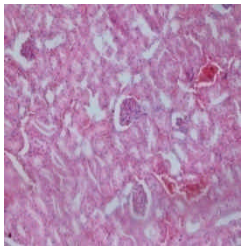
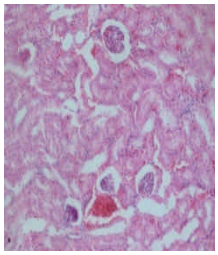
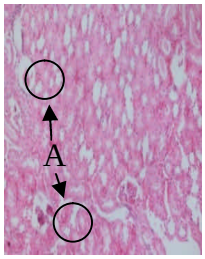
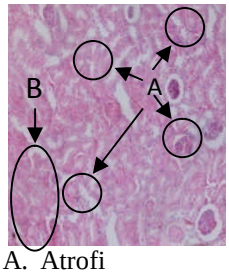
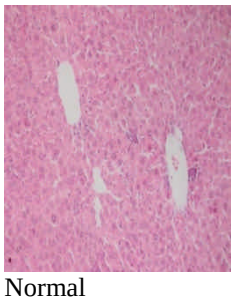
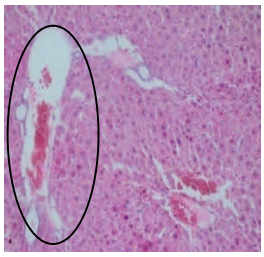
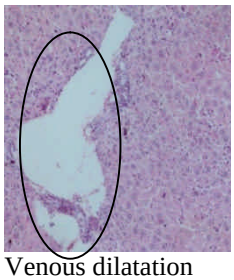
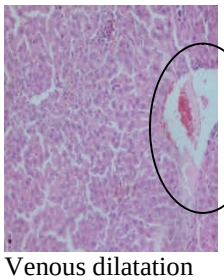
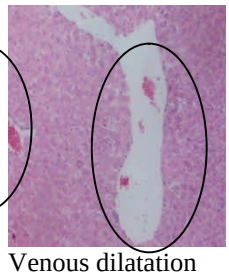
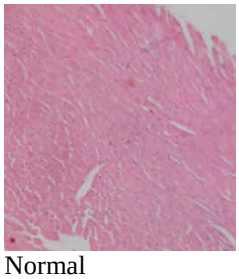
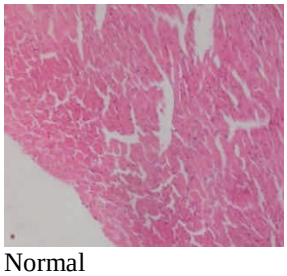
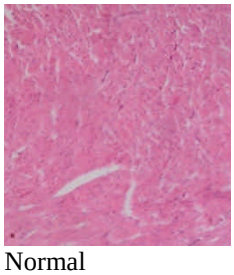
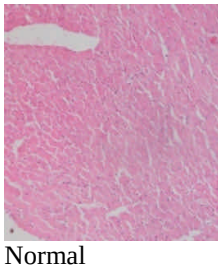
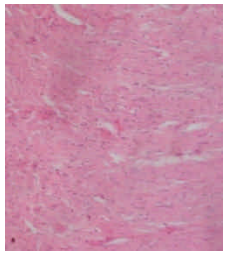
Description: Regresion equation: y = -1.088+1.39x

Based on the result of histopathology, liver and heart looked normal, but there is atrophy of the glomeruli in the kidneys of two mice (8000 mg/kg treatment groups). These degenerative changes are an early manifestation of cell injury. This incident is reversible because it seen improvement in cells. However, if injuries persist, so the necrosis will occur. There is no significant change in pathological effects of all tested dose levels during the observation period of 14 days.

Conclusions

All of the tested group have significant diuretic activity ($p<0.05$) in comparison with control normal group. A single dose of *E. longifolia* extract (40 mg/kgBW) and irbesartan (dose: 40 mg/kgBW) had no

Table 3 Results of Histopathology

Control	Doses 1 g	Doses 2 g	Doses 4 g	Doses 8 g
Kidney				
 N. Normal glomerulus	 N. Normal glomerulus	 N. Normal glomerulus	 A. Atrofi glomerulus	 A. Atrofi glomerulus B. Oedema tubulus
Liver				
 Normal		 Venous dilatation	 Venous dilatation	 Venous dilatation
Heart				
 Normal	 Normal	 Normal	 Normal	 Normal

significant difference. But the combination of *E. longifolia* extract and irbesartan are lower activity than single extract.

Based on table toxicity scale, LD_{50} value of combination *E. longifolia* extract with irbesartan is 23.981 g/kg and can be categorized as nontoxic compound. The histopathological result showed that there is no significant pathologic effect in the kidneys, liver, and heart.

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