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Article

Effect of *Muntingia Calabura* L. Stem Bark Extracts on Uric Acid Concentration and Renal Histopathology in Diabetic Rats

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Abstract: *Background and objectives:* This study were designed to determine uric acid concentration and renal histopathology of *M. calabur*a L. stem bark extract in diabetic rats and to compare the natural product of *M. calabura* L. stem bark extract with allopurinol. *Materials and Methods:* A completely randomized design was used for the experiment which consisted of 6 treatment groups, each consisting of 4 rats, as follows: 1) NR, normal rat; 2) KN, diabetic rat (negative control); 3) KP, diabetic rats given allopurinol 10 mg/kg body weight; 4) EM150, diabetic rats given the test extract 150 mg/kg body weight/day; 5) EM300, diabetic rats given the test extract 300 mg/kg body weight/day; and 6) EM450, diabetic rats given the test extract 450 mg/kg body weight/ day. *Results:* The results showed that *M. calabura* L. stem bark extract decrease (p<0.05) uric acid levels in diabetic rats and no specific damage to renal proximal tubular cells was seen. *Conclusions:* It was concluded that *M. calabura* L. stem bark extract has a potential as an antihyperuricemic in diabetic rats. The recommended does was 300 mg/kg body weight to provide a significant effect on reducing the uric acid level in diabetic rats. Our findings support the use of this plant as a treatment for gout and other inflammatory diseases.

Keywords: uric acid levels; aloxan; diabetic rat; *Muntingia calabura* L.

1. Introduction

Diabetes mellitus, defined by elevated glycemic markers, is a major risk factor for cardiovascular disease, which is the most common cause of death among adults with diabetes mellitus [1]. In diabetic patients, hypertension and decreased renal function with hyperuricemic are major problems [2]. Hyperuricemia means high levels of uric acid in the blood, a condition considered to be closely associated with increased risks for developing gout, cardiovascular diseases, hypertension, and metabolic syndrome [3,4]. Since diabetic is often complicated by hypertension and hyperuricemic, efficient therapeutic strategy against these two complications is very important in the treatment of diabetes. A study showed that decreases serum uric acid level in patients with diabetes [5]. Uric acid has been linked with increased risk of chronic disease such as cardiovascular disease, and this association has been attributed to a inflammatory effect [6]. Antihyperuricemic drug can be further explored not only as antigout therapeutics but also in other systems where hyperuricemic is the driving cause of the disease [7].

Diabetic rats have an intracardiac cytokine protein expression profile that is reflective of a weaker cardiac host defense compared with that seen in the healthy rat. Elevation of serum uric acid levels, a surrogate marker for heart disease, was suppressed to comparable levels to that in healthy rats [8].

Currently, hyperuricemia is treated by using natural ingredients from the *Muntingia calabura* L. plant., a flowering plant, in the province of Aceh. It contains polyphenols, flavonoids, ascorbic acid,

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α - tocopherols, and triterpenoids [9]. The leaves of the cherry plant (*Muntingia calabura* L.) are reported to have anti-inflammatory and antioxidant properties [10]. An antioxidant has the ability to reduce blood sugar level [11]. An antioxidant could reduce serum uric acid level [12]. Normally the use of synthetic drugs cause kidney organ disorders, this study evaluates the effect of natural ingredients *M. calabura* on kidney histopatology. The antihyperuricemic potential of the ethanol extract of *M. calabura* L. stem bark from Aceh in diabetic rats has not yet been reported.

This study was designed to determine the potential of *M. calabura* L. stem bark extracts from Aceh an antihyperuricemic in diabetic rats and to compare the natural *M. calabura* L. stem bark extract with allopurinol.

2. Materials and Methods

2.1. Preparation of ethanol extract of M. calabura L.

M. calabura L. stem bark was collected in the mountainous region Aceh Besar of Aceh province, Indonesia. The plant was authenticated by the Department of Biology Education, Syiah Kuala University. The dried plant material (5 kg) was extracted three times with 96% ethanol at room temperature for 3 days. The solution was filtered and combined, and the organic solvent was removed under reduced pressure to give a crude ethanol extract. The filtrate was vacuum evaporated at low temperature (40 °C) until a semi solid residue was obtained. The crude extract of each part was dried by placement in a vacuum desiccator [13,14].

2.2. Qualitative phytochemical screening

The crude extract was qualitatively tested for the presence of chemical constituents by performing various tests such as Mayer's test for alkaloid, Salkowski's test for steroids, aluminum chloride test for flavonoids, and ferric chloride test for phenolic compounds. These were identified by the characteristic color change using the standard tests [15,16,17].

2.3. In vivo experiment

The adult male Wistar rats (8 weeks old, weighing 180 g) were obtained from Animal Facilities, Research Center for Medicine and Pharmacy, Syiah Kuala University, Banda Aceh (Aceh, Indonesia). Animals were used according to the suggested ethical guidelines for the care of laboratory animals, and the experimental protocols used in this study were approved by the Scientific and Ethical Committee of Syiah Kuala University. The rats were acclimatized for at least 7 days to adapt to their environment before any experimental manipulation. They were housed in an animal room (Department of Pharmacology, Syiah Kuala University) with a temperature of 24°C–26°C, humidity of 55%–60%, a regular 12/12h light/ dark cycle (7:00a.m.–7:00p.m.), and free access to standard laboratory diet and tap water until used for the experiments. General health status of the rats was monitored on alternate days, and no adverse events were recorded during the housing period. At the beginning of each experiment, the body weight of the animals ranged from 180 to 220 g. The completely randomized design was used for the experiment which consisted of 6 treatment groups, each consisting of 4 rats, as follows: 1) NR, normal rat; 2) KN,diabetic rat (negative control); 3) KP, diabetic rats given allopurinol 10 mg/kg body weight;, 4) EM150, diabetic rats given the test extract 150 mg/kg body weight/day; 5) EM300, diabetic rats given the test extract 300 mg/kg body weight/day; and 6) EM450, diabetic rats given the test extract 450 mg/kg body weight/day. Diabetes was induced in the animals by intra peritoneal injection of freshly prepared aloxan in a single dose of 150 mg/kg. One week after aloxan injection, fasting blood glucose was measured to verify the development of diabetes. The *M. calabura* L. stem bark extract was given for 45 days. Body weight, naso-anal length, and body mass index were measured as described by Ahmed et al. [18]. The crude ethanol extract from *M. calabura* L. stem bark at 150, 300, and 450 mg/kg were administered orally for 5 days to aloxan-induced diabetic rats, and serum uric acid levels were measured by Gluco check "Nesco". Blood uric acid level was monitored with the Nesco Multi check (Gesunde Medical, Indonesia).

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2.4. Histopathological determination

For microscopic evaluation, tissues were fixed in a fixative (neutral buffered formalin) and embedded in paraffin, sectioned at 4µm, and subsequently, stained with hematoxylin-eosin [19,20,21,22]. Sections were studied under a light microscope (DP12 Olympus) at 40 magnifications. Slides of all the treated groups were studied and photographed. A minimum of 12 fields of each section was studied and approved by a pathologist who did not know the treatment given.

2.5. Statistical data analysis

Data are expressed asthemean ± standard deviation (SD). Statistical analysis was performed by one-way analysis of variance. Duncan's multiple range test was performed to determine significant differences among the means. The values of p <0.05 were considered to be statistically significant.

3. Results

3.1. Phytochemical screening

Qualitative phytochemical screening of the *M. calabura* stem bark extract shows the presence offlavonoids, alkaloid,triterpenoid, steroid, and poliphenolic compounds.

3.2. Effect of crude ethanol extract from M. calabura L. stem bark on body weight and body mass index

Administration of *M. calabura* L. stem bark extract after 45 days of treatment had an effect on the body weight. Body weight of rats which givenn the stem bark extract was the same as that of normal rats. However, the body weight in diabetic rats was lower when compared with other treatment groups. Administration of *M. calabura* L. stem bark extract had no effect on the body mass index (Table 1).

The crude ethanol extract from *Muntingia calabura* L.stem barkat 150, 300, and 450 mg/kg and allopurinol at 10 mg/kg were administrated orally once a day. The control diabetic rat and normal groups. Values are displayed as mean ± SD. The super script letters in the same row indicate statistically significant values (p<0.05).

3.3. Effect of crude ethanol extract from M. calabura L. stem bark on serum uric acid levels in rats

The crude ethanol extract from *M. calabura* L.stem bark at 150, 300, and 450 mg/kg were administered orally for 5 days to aloxan-induced diabetic rats, and serum uric acid levels were measured by Gluco check "Nesco". As presented in Table 1,compared with the uric acid levels in the normal rats (4.675 mg/dL), the levels in the control diabetic rats (10.225 mg/dL) increased significantly (p<0.05). Administration of the crude ethanol extract from *M. calabura* L. stem bark at 150, 300, and 450 mg/kg could reduce theuric acid levels (p<0.05) compared with the diabetic rats. The positive control which was administered allopurinol at a dose of 10 mg/kg displayed hypouricemic activity which significantly reduced the serum uric acid level, and same was seen with the rats given the crude ethanol extract from *M. calabura* L. stem bark at 300 and 450 mg/kg (Table 2).

The crude ethanol extract from *Muntingia calabura* L. stem barkat 150, 300, and 450 mg/kg and all opurinol at 10 mg/kg were administrated orally once a day. The control diabetic rat and normal groups were orally administered with aquadest. Values are displayed as mean±SD. The superscriptletters in the same row indicate statistically significant values (p<0.05).

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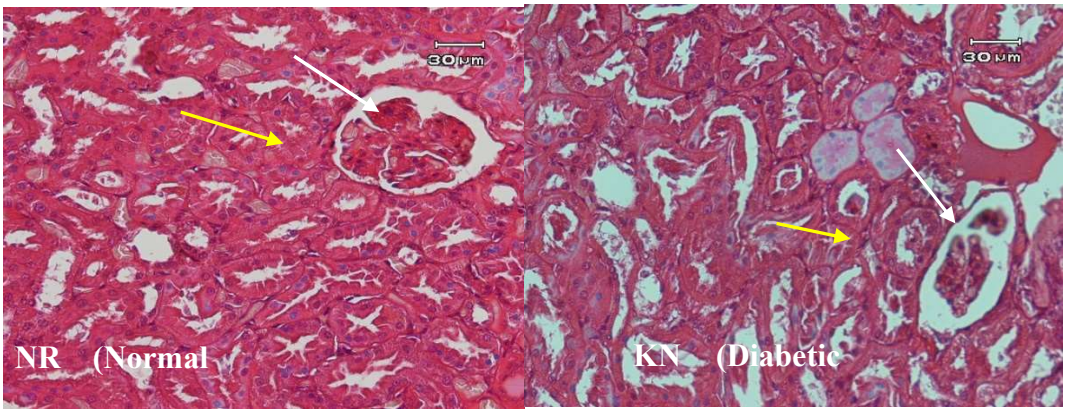
133 **Table 1.** Body weight and BMI of rats on various treatments.

Treatment	Body weight (g)	Body mass index (g/cm²)
NR (Normal rat)	213±6.05[a]	0.44±0.18[a]
KN (Diabetic rat)	169±8.20[d]	0.42± 0.02[a]
KP (Allopurinol)	188±9.55[c]	0.47±0.02[a]
EM150	200.5±6,65[b]	0.50±0.01[a]
EM300	208.25±5.5[ab]	0.52±0.01[a]
EM450	201.75±7.13[ab]	0.50±0.01[a]

134 **Table 2.** Inhibitory effect of crude ethanol extract from *M. calabura* L. stem bark on serum uric.acid
135 levels in rats.

Treatment	Serum uric acid levels (mg/dL)	Inhibition (%)
NR (Normal rat)	4.675 ± 2.01 [b]	
KN (Diabetic rat)	10.225 ± 1.88 [a]	
KP (Allopurinol)	5.000 ± 1.29 [b]	51.10
EM150	7.025 ± 3.33 [ab]	31.29
EM300	5.125 ± 1.77 [b]	50.02
EM450	6.600 ± 2.54 [b]	35.45



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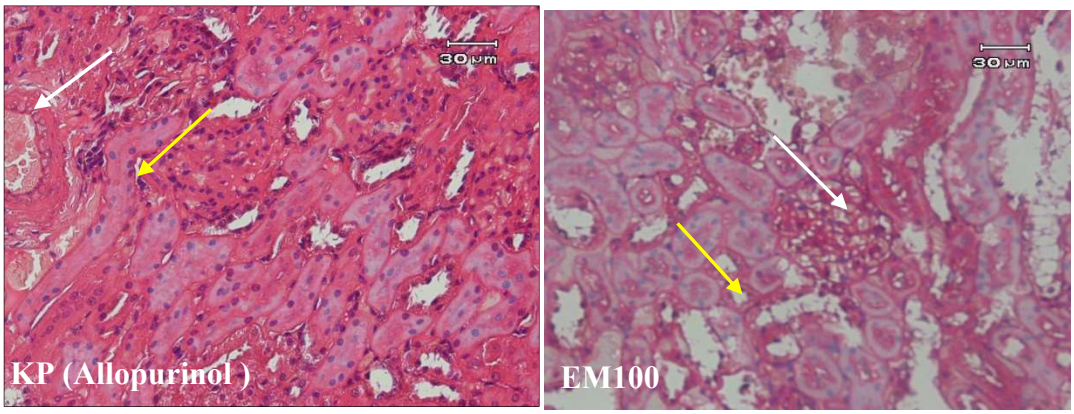

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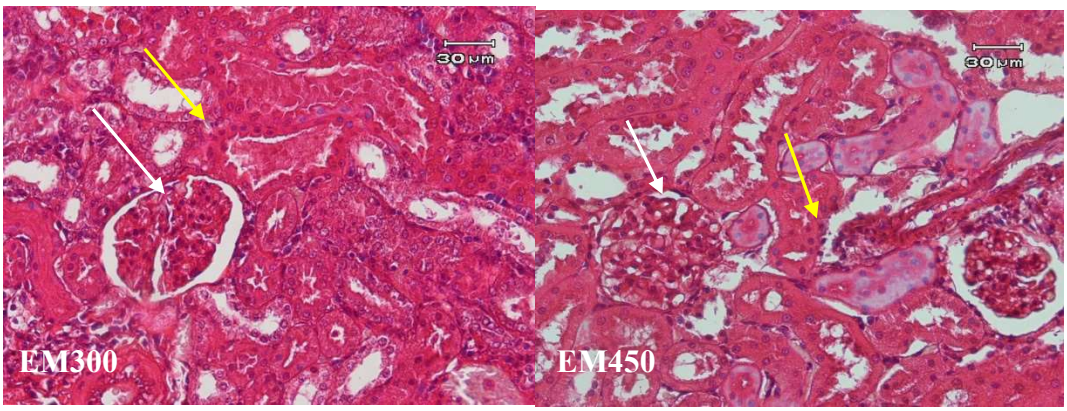

Figure 1. Histopathological studies of the kidney. Histological examination of kidney in rats which were administered the crude ethanol extract from *Muntingia calabura* L. stem barkat 150, 300, and 450 mg/kg and allopurinol at 10 mg/kg once a day. The control diabetic rat and normal groups were orally administered with aquadest. Yellow arrow, proximal convoluted tubule; white arrow, glomerulus.

4. Discussion

The ethanol extract from *M. calabura* L.stem bark at 300 mg/kg displayed the same inhibitory effect on serum uric acid levels as in ratswhich received allopurinol (Table 2).The presence of flavonoid sand poliphenolic compounds in the *M. calabura* stem bark extract may have reduced uric acid in the rats. Chen-Yu et al. [23] reported that flavonoids in *Davallia formosana* extract can decrease uric acid levels by inhibition of xanthine oxidase enzyme. In particular, plant phenolic compounds, such as phenolic acids and flavonoids, exhibit strong antioxidant activities via scavenging free radicals. Moreover, many studies have also indicated that both types of compounds inhibited xanthine oxidase activity [24,25,26].

The results of these trials are consistent with the reports indicating favorable effects of serum uric acid lowering treatment with allopurinol on the rate of cardiovascular complications in patients with coronary heart disease, congestive heart failure, and dilated cardiomyopathy [27]. The crude ethanol extract of *Siegesbeckia orientalis* displayed antihyperuricemic activity, and the *n*-butanol-soluble fraction was found to be the most active portion of the extract. Further,*in vivo* studies of this fraction showed 31.4% decrease of serum uric acid levels [13]. Antihyperuricemic effect of novel thiadiazolopyrimidin-5-one analogs has been showed in oxonate treated rats, which can be further explored not only as antig out therapeutics but also in other systems where hyperuricemia is the driving cause of the disease [7]. The results indicated that activity of phytochemicals from *D. formosana* significantly inhibited xanthine oxidase activity *in vitro* and reduced serum uric acid levels *in vivo*. This is the first report providing new insights into the antihyperuricemic activities of flavonoid glycosides which can possibly be developed into potential hypouricemic agents [23].

The results of renal histopathology showed that administration of *M. calabura* L. stem bark extract improved the nucleus of proximal renal tubular cells and showed no specific damage to renal proximal tubular cells (Figure 1). in uncomplicated obese, insulin-resistant and hypertensive patients serum uric acid levels increase mainly as a consequence of impaired renal excretion, in conditions of local ischemia an increased production of uric acid occurs in parallel with that of reactive oxygen species [27]. Body Mass Index (BMI) has an effect on Diabetes. BMI levels were positively correlated with Plasminogen-activator network (t-PA Ag), as well as Plasminogen Activator Inhibitor-1 (PAI-1 Ag) concentration, and BMI can be associated with high plasma levels of PAI-1 Ag in Type 2 Diabetes [28].

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5. Conclusions

It was concluded that *M. calabura* L. stem bark extract decrease uric acid levels and no specific damage to renal proximal tubular cells, has potential as antihyperuricemics in diabetic rats. The recommended does to provide a significant effect on reducing the uric acid level in diabetic rats is 300 mg/kg body weight.

Author Contributions: S .S. conceived and design of the research, acquisition of the data, analysis and interpretation of the data, drafting of the manuscrips and its revision, agree and final approval of the version to be submitted, and submit the journal and M. S. conducted the experiment. All authors read and approved the manuscript.

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Conflicts of Interest: The authors declare that they have no conflicts of interest.

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