

Nephroprotective Effect of Ethanolic Extract of Balakka (*Phyllanthus emblica* L.) on Rats Induced Ethylene Glycol and Ammonium Chloride

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Abstract. The kidneys are a pair of symmetrical organs that function to filter the blood and are located in the retroperitoneal region on the posterior wall of the abdomen. The kidneys are drained about 25% of cardiac output. Kidney damage due to the accumulation of kidney stones can be caused by ethylene glycol substances to form calcium oxalate crystals (CaC_2O_4). This crystal is common in urine specimens even in healthy animals. Crystal formation can be accelerated by the administration of ammonium chloride. This study aims to determine the nephroprotective effects of balakka ethanol extract to reduce creatinine and urea levels in rats induced by ethylene glycol and ammonium chloride. The results showed that the balakka ethanol extract with a dose variation of 50 mg/kg body weight, 100 mg/kg body weight, and 150 mg/kg body weight had nephroprotective activity in rats that had been induced by ethylene glycol and ammonium chloride. Where the induction group had the largest serum creatinine mean of 1.43 mg/dl. The ethanolic extract of balakka (EEB) treatment group dose of 50 mg/kg BW had a serum creatinine value of 1.01 mg/dl. The EEB treatment group dose of 100 mg/kg BW had a serum value of 0.91 mg/dl. The balakka ethanol extract treatment group dose of 150 mg/kg BW had a serum value of 0.73 mg/dl. The induction group had the largest serum urea serum of 76.56 mg/dl. EEB treatment group dose of 50 mg/kg BW had a serum urea value of 63.36 mg/dl. The EEB treatment group dose of 100 mg/kg BW had a serum urea value of 49.73 mg/dl. The balakka ethanol extract treatment group dose of 150 mg/kg BW had a serum urea value of 49.90 mg/dl.

Key Word : ureum, creatine, kidney.

Abstrak. Ginjal adalah sepasang organ simetris yang berfungsi untuk menyaring darah dan terletak di daerah retroperitoneal di dinding posterior perut. Ginjal dikuras sekitar 25% dari curah jantung. Kerusakan ginjal akibat penumpukan batu ginjal dapat disebabkan oleh zat etilen glikol untuk membentuk kristal kalsium oksalat (CaC_2O_4). Kristal ini umum dalam spesimen urin bahkan pada hewan yang sehat. Pembentukan kristal dapat dipercepat dengan pemberian amonium klorida. Penelitian ini bertujuan untuk mengetahui efek nefroprotektif dari ekstrak etanol balakka untuk mengurangi kadar kreatinin dan urea pada tikus yang diinduksi oleh etilen glikol dan amonium klorida. Hasil penelitian menunjukkan bahwa ekstrak etanol balakka dengan variasi dosis 50 mg / kg berat badan, 100 mg / kg berat badan, dan 150 mg / kg berat badan memiliki aktivitas nefroprotektif pada tikus yang diinduksi oleh etilen glikol dan

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amonium klorida. Di mana kelompok induksi memiliki rata-rata kreatinin serum terbesar 1,43 mg / dl. Ekstrak etanol dari kelompok perlakuan balakka (EEB) dosis 50 mg / kg BB memiliki nilai kreatinin serum 1,01 mg / dl. Kelompok perlakuan EEB dosis 100 mg / kgBB memiliki nilai serum 0,91 mg / dl. Kelompok perlakuan ekstrak etanol balakka dosis 150 mg / kgBB memiliki nilai serum 0,73 mg / dl. Kelompok induksi memiliki serum urea serum terbesar 76,56 mg / dl. Kelompok perlakuan EEB dosis 50 mg / kgBB memiliki nilai urea serum 63,36 mg / dl. Kelompok perlakuan EEB dosis 100 mg / kgBB memiliki nilai urea serum 49,73 mg / dl. Kelompok perlakuan ekstrak etanol balakka dosis 150 mg / kgBB memiliki nilai urea serum 49,90 mg / dl.

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1. Introduction

Impaired kidney function can be caused by several factors, including can be caused by hypertension, the presence of blockages in the urinary tract, autoimmune disorders, urinary tract infections, and diabetes mellitus. Impaired kidney function due to an accumulation of kidney stones can also be caused by ethylene glycol substances to form calcium oxalate crystals (CaC_2O_4). This crystal is common in urine specimens even in healthy animals. Crystal formation can be accelerated by the administration of ammonium chloride. Crystals usually form in urine which has an acidic pH and can appear in urine specimens after an animal experiences ethylene glycol poisoning or consumes certain foods such as asparagus and cabbage [1].

The administration of compounds that can protect the kidneys (nephroprotective) from the toxic effects of ethylene glycol and ammonium chloride is very necessary. Antioxidant compounds such as curcumin, quercetin, and taurine are known to improve kidney function that is damaged by paracetamol [2-4].

The methods used to treat kidney disorders include the method of hemodialysis, peritoneal dialysis, and kidney transplantation. However, the best method of fighting kidney disease is of course through prevention. Prevention that is commonly done by the community includes doing a healthy lifestyle. A healthy lifestyle is done such as drinking lots of water every day, exercising, healthy eating patterns and what is currently popular in the community is by taking natural supplements in the form of traditional plants. Commonly used plants for treating kidney disease include meniran leaves, tempuyung leaves, keji beling leaves, senggani leaves and balakka fruits [5].

Balakka is a material that is often used by the community as traditional medicine. This plant in India has been used to treat cancer, diabetes, liver (liver), heart problems and anemia [6]. The phytochemical analysis shows that balakka has alkaloid compounds, phenols (flavonoids, tannins, saponins) and steroids in leaves, bark, and fruit. The highest secondary metabolite content found in all three balakka organs is tannins from the phenol group [7].

Balakka (*Phyllanthus emblica L.*) is a common type of plant that has various properties for human health, but herbs have not been used optimally as a source of herbal medicine. The content of flavonoids (kaempferol apigenin, luteolin) is found in balakka leaves which are expected to dissolve calcium oxalate salts. This is the basis of the importance of this research, given the prevalence of kidney stone disease is increasing year by year according to a survey from the Ministry of Health of the Republic of Indonesia 2017. This study aims to determine the nephroprotective effects of balakka ethanol extract in reducing creatinine and urea levels in rats induced by ethylene glycol and ammonium chloride [8-10].

2. Method

This type of research is experimental research that is to determine the effect or relationship of independent variables on the dependent variable. The independent variable is balakka fruit extract (*Phyllanthus emblica L.*), while the dependent variable is the nephroprotective effectiveness of balakka fruit extract. This research was conducted at the Pharmacy Pharmacology Laboratory, University of North Sumatra.

2.1 Materials

The sample used in this study was balakka obtained from South Tapanuli, North Sumatra. The test materials used in this study were balakka fruit extract (*Phyllanthus emblica L.*), aquades, demineralized fish, urea reagents, creatinine reagents, alpha-naphthol, amyl alcohol, acetic anhydride, concentrated hydrochloric acid, concentrated nitric acid, sulfuric acid concentrated, benzene, iron (III) chloride, bismuth (III) nitrate, dimethyl sulfoxide (DMSO), ethanol, ethyl acetate, iodine, isopropanol, potassium iodide, chloroform, crystals of sodium hydroxide, methanol n-hexane, sodium chloride, mercury (II) chloride, lead (II) acetate, magnesium powder, chloral hydrate, ethylene glycol, ammonium chloride, ammonium oxalate, nitric acid, formalin buffer, and hematoxylin and eosin,

2.2 Animals

Animals used in research is a rat (*Rattus norvegicus*) Wistar male 150 – 200 g. Before the study began, animal test adjusted for one week with the condition of the room temperature (22-25 ° C), under the cycle of 12 hours light/ dark, given the food and the drinking water ad libitum [11]. Ethics Commission from health and science commission, University of Sumatera Utara

2.3 In vivo test nephroprotective effect of balakka ethanol extract

In vivo tested in an experiment by using 25 Wistar rats (*Rattus norvegicus*) male and weight 150 g - 200 g, as many as 25 and divided into 5 groups and each group consisted of 5 rats :

Normal	: Suspension Na-CMC (normal)
Negative control	: Wistar rats (<i>Rattus norvegicus</i>) male induced by etilen glycol 0.75% and ammonium chloride 2%
Dose I	: Wistar rats (<i>Rattus norvegicus</i>) male induced by etilen glycol 0.75% and ammonium chloride 2% + extract 50 mg/kg body weight
Dose II	: Wistar rats (<i>Rattus norvegicus</i>) male induced by etilen glycol 0.75% and ammonium chloride 2% + extract 100 mg/kg body weight
Dose III	: Wistar rats (<i>Rattus norvegicus</i>) male induced by etilen glycol 0.75% and ammonium chloride 2% + extract 150 mg/kg body weight

Induction of kidney stones by administering 0.75% ethylene glycol and 2% ammonium chloride as much as 1% of the body weight of the rat orally. Before the treatment, the mice were adapted for 14 days then followed by giving induction for 10 days and the treatment of experimental animals for 10 days. On the 11th day, the rats were treated each group continued until day 20 [12]. The 21st day of the experimental animal was decanted by neck dislocation. Rat blood was taken to test creatinine and urea levels.

3. Result and Discussion

3.1 Result of Creatinine

In this study, serum creatinine was examined from the blood of mice. Serum creatinine examination was carried out in the Medan area health laboratory. The serum creatinine results obtained can be seen in table 1.1 below:

Table 1. Level of creatinine

No.	Groups	Creatinine $\pm$ SD (mg/dl)
1.	Normal group	0.79 ± 0.02
2.	Induction only group	1.43 ± 0.05
3.	Treatment group 50 mg/kg body weight of extract	1.02 ± 0.02
4.	Treatment group 100 mg/kg body weight of extract	0.91 ± 0.03
5.	Treatment group 150 mg/kg body weight of extract	0.73 ± 0.02

Based on the table it is known that the average serum creatinine value for the normal group is still within the normal range of 0.79 mg/dl.

The Induction group had the largest serum creatinine level of 1.43 mg/dl. The balakka ethanol extract treatment group dose of 50 mg / kg body weight had a serum creatinine value of 1.01 mg/dl. The balakka ethanol extract treatment group dose of 100 mg / kg body weight had a serum value of 0.91 mg/dl. The balakka ethanol extract treatment group dose of 150 mg / kg body weight had a serum value of 0.73 mg/dl.

Based on the table, it is known that the average serum creatinine in the largest treatment group is 1.01 mg/dl at the EEB dose of 50 mg / kg body weight. And the average serum creatinine in the smallest treatment group was 0.73 in the balakka ethanol extract dose of 150 mg / kg body weight. In addition, it can be seen that there is a decrease in serum creatinine levels as the balakka ethanol extract dose increases.

Based on the results of statistical tests, the serum creatinine levels of the normal group had a significant difference ($p < 0.05$) with the treatment group of the induction group and did not have a significant difference ($p > 0.05$) with the EEB treatment group dose of 50 mg/kg BW, the EEB treatment group dose 100 mg/kg BW, and EEB treatment group dose 150 mg/kg BW. The induction group had a significant difference ($p < 0.05$) with the normal group, the treatment group dose 50 mg/kg BW, the treatment group dose 100 mg/kg BW, and the treatment group dose 150 mg/kg BW. The treatment group dose of 50 mg/kg BW had a significant relationship ($p < 0.05$) with the induction group, did not have a significant relationship ($p > 0.05$) with the normal group, treatment dose of 100 mg/kg BW, and treatment dose of 150 mg/kg BW. The treatment group dose of 100 mg/kg BW had a significant relationship ($p < 0.05$) with the induction group, did not have a significant relationship ($p > 0.05$) with the normal group, treatment dose 50 mg/kg BW, and treatment dose of 150 mg/kg BW. The treatment group dose of 150 mg/kg BW had a significant relationship ($p < 0.05$) with the induction group, did not have a significant relationship ($p > 0.05$) with the normal group, treatment dose 50 mg/kg BW, and treatment dose of 100 mg/kg BW [13,14].

3.2 Result of Ureum Level

In this study, serum urea was examined from the blood of mice. Examination of serum urea was carried out in the Medan area health laboratory. The serum results obtained can be seen in table 2 below:

Table 2. Ureum level

No.	Groups	Ureum $\pm$ SD (mg/dl)
1.	Normal group	45.43 $\pm$ 2
2.	Induction only group	76.56 $\pm$ 4.04
3.	Treatment group 50 mg/kg body weight of extract	63.36 $\pm$ 3.05
4.	Treatment group 100 mg/kg body weight of extract	49.73 $\pm$ 1.52
5.	Treatment group 150 mg/kg body weight of extract	49.90 $\pm$ 2.51

Based on the table it is known that the average serum urea value for the normal group is still in the range of the normal value of 45.43 mg/dl.

The induction group had the largest serum urea serum of 76.56 mg/dl. EEB treatment group dose of 50 mg/kg BW had serum urea value of 63.36 mg/dl. The EEB treatment group dose of 100 mg/kg BW had a serum urea value of 49.73 mg/dl. The EEB treatment group dose of 150 mg/kg BW had a serum urea value of 49.90 mg/dl.

Based on the table it is known that the average serum urea in the largest treatment group is 63.36 mg/dl at EEB administration at a dose of 50 mg/kg BW. And the average serum creatinine in the smallest treatment group was 49.90 at EEB doses of 150 mg/kg BW. In addition, it can be seen that there is a decrease in serum creatinine levels as the EEB dose increases.

Based on the results of statistical tests, serum urea levels in the normal group had a significant relationship ($p < 0.05$) with the induction group and did not have a significant relationship ($p > 0.05$) in the treatment group dose of 50 mg/kg BW, the treatment group dose was 100 mg/kg BW, and the treatment group dose 150 mg / kg. The induction group had a significant relationship ($p < 0.05$) with the normal group, the treatment group dose was 50 mg/kg BW, the treatment group dose was 100 mg/kg BW, and the treatment group was 150 mg/kg BW. The treatment group dose of 50 mg / kg BW had a significant relationship ($p < 0.05$) with the induction group, and did not have a significant relationship ($p > 0.05$) with the normal group, the treatment group dose of 100 mg / kg, and the treatment group dose of 150 mg/kg BW. The treatment group dose of 100 mg/kg has a significant relationship ($p < 0.05$) with the induction group, and does not have a significant relationship ($p > 0.05$) with the normal group, the treatment group dose 50 mg/kg, and the treatment group dose of 150 mg/kg BW. The treatment group with a dose of 150 mg/kg had a significant relationship ($p < 0.05$) with the induction group, and did not have a significant relationship ($p > 0.05$) with the normal group, treatment group dose 50 mg/kg, and treatment group dose 100 mg/kg BW [15].

4. Conclusions

Ethanol extract of balakka fruit dose of 50 mg/kg bw, 100 mg/kg bw and 150 mg/kg bw had nephroprotective activity on male rats induced by ethylene glycol and ammonium chloride. The effective dose of balakka ethanol extract as nephroprotective was at a dose of 150 mg / kg bw with serum urea level 49.90 ± 2.51 mg/dl and serum creatinine level 0.73 ± 0.02 mg/dl which showed a significant difference ($p < 0.05$) from the group induction and did not differ significantly ($p > 0.05$) from the normal group and did not show any damage

to kidney tissue on tissue histopathology examination. Ethanol extract of balakka fruit dose of 50 mg/kg bw, 100 mg/kg bw and 150 mg/kg bw had nephroprotective activity on male rats induced by ethylene glycol and ammonium chloride. The effective dose of balakka ethanol extract as nephroprotective was at a dose of 150 mg / kg bw with serum urea level 49.90 ± 2.51 mg/dl and serum creatinine level 0.73 ± 0.02 mg/dl which showed a significant difference ($p < 0.05$) from the group induction and did not differ significantly ($p > 0.05$) from the normal group and did not show any damage to kidney tissue on tissue histopathology examination.

REFERENCES

- [1]. S. Abedon and C. Thomas-Abedon, "Phage Therapy Pharmacology," *Current Pharmaceutical Biotechnology*, vol. 11, no. 1, pp. 28–47, Jan. 2010.
- [2]. W.F. Ganong, W. F, *Review of medical physiology*. Mcgraw-hill.1995
- [3]. O.P. Heinonen, and D. Albanes, "The Effect of Vitamin E and Beta Carotene on the Incidence of Lung Cancer and Other Cancers in Male Smokers," *New England Journal of Medicine*, vol. 330, no. 15, pp. 1029–1035, Apr. 1994.
- [4]. E. Hodgson, , *A textbook of Modern Toxicology*, Fourth Edition, John Wiley&Sons, Inc, Canada, pp. 292- 294. 2010
- [5]. B.G Katzung, *Basic and clinical pharmacology*. McGraw-Hill Education.2017
- [6]. X. Liu, M. Zhao, J. Wang, B. Yang, and Y. Jiang, "Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China," *Journal of Food Composition and Analysis*, vol. 21, no. 3, pp. 219–228, May 2008.
- [7]. N.A Lumenta, *Kidney Stone disease and urinary tract: Causes and Therapy*. Editor team: Susalit, E., dkk. Jakarta: PT BPK Gunung Mulia, 1992
- [8]. B.G. Katzung, *Pharmacology: Basic and clinic*, Buku 2. Edisi I, Salemba Medika, Jakarta, 2002
- [9]. W.F. Ganong, *Review of medical physiology*. Mcgraw-hill.1995
- [10]. N. Çiftçioglu, M. Björklund, K. Kuorikoski, K. Bergström, and E. O. Kajander, "Nanobacteria: An infectious cause for kidney stone formation," *Kidney International*, vol. 56, no. 5, pp. 1893–1898, Nov. 1999.
- [11]. C.P Porth, G. Matfin, *Pathophysiology : Concept of Altered Health States*, Eight Edition, Lippincott Williams & Wilkins, USA, 2009.
- [12]. Price, S.A., and Wilson, L.M., 1985, *Pathophysiology Clinical Concepts of Disease Processes*, diterjemahkan oleh Dharma, A., Penerbit Kedokteran EGC, Jakarta, 1985
- [13]. Robbins and Cotran, *Pathologic Basis of Disease*, Edisi 7, Elsevier Inc, New York, USA, 2007
- [14]. D. F. Kelton, K. D. Lissemore, and R. E. Martin, "Recommendations for Recording and Calculating the Incidence of Selected Clinical Diseases of Dairy Cattle," *Journal of Dairy Science*, vol. 81, no. 9, pp. 2502–2509, Sep. 1998.
- [15]. N. S. Mandel and G. S. Mandel, "Urinary Tract Stone Disease in the United States Veteran Population. II. Geographical Analysis of Variations in Composition," *Journal of Urology*, vol. 142, no. 6, pp. 1516–1521, Dec. 1989.