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Research Article

EFFECT OF ETHANOLIC EXTRACT OF BREADFRUIT (*ARTOCARPUS ALTILIS* [PARKINSON] FOSBERG) LEAVES ON AMELIORATING RENAL FUNCTION OF RAT

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ABSTRACT

Aims: Kidneys are one of the vital organs which belong to the urinary system. Renal failure occurs when the kidneys are unable to perform excretory functions which are important in maintaining homeostasis. Breadfruit leaves are commonly used empirically for the treatment of renal failure.

Objectives: This study aims to determine the influence of breadfruit leaves ethanol extract (BLEE) to improve renal function in the renal failure rat model.

Methods: Renal failure model was conducted by administering gentamicin 100 mg/kg body weight (bw) intraperitoneally for 7 days consecutively and piroxicam 3.6 mg/kg bw for 5 weeks which was starting administered with gentamicin. BLEE were given for 4 weeks with dose of 50, 100, and 200 mg/kg bw, after gentamicin induction. Renal functions were evaluated by measuring urea and creatinine serum levels every week and renal histology at the end of the experiment.

Results: After induction, urea and creatinine serum levels increased up to 5 times ($p<0.05$). Both parameters in all groups went up until the 2nd week and dropped on the 3rd week. There was a significant difference in urea and creatinine serum levels in rats treated by BLEE 200 mg/kg. Histological results depicted an improvement of the kidney structure. That treated by BLEE 200 mg/kg showed similarities appearance with the normal.

Conclusion: BLEE improved the kidney function in the renal failure model which was showed by reduction on the levels of serum urea and creatinine and restore kidneys structure. BLEE 200 mg/kg bw showed better activity on ameliorating renal function.

Keywords: Renal failure, Serum urea, Serum creatinine, Breadfruit leaves, Gentamicin, Piroxicam.

INTRODUCTION

The kidneys are very important organs which exhibit crucial functions in the body, providing an excretory mechanism as part of the urinary tract to eliminate waste of metabolism and so on. Besides, it plays an important role in maintaining homeostasis [1]. Disorder in the kidneys will lead to disturbance of fluid and electrolyte. The kidney may lose its ability to preserve biochemical balance, cause retention of the waste compound, fluid imbalance, and acid-base disturbance. Such disorders may further involve in renal failure [2].

Renal failure is divided into two types: Acute and chronic. Acute kidney failure is a potentially life-threatening clinical syndrome, but it occurs reversibly. It may happen primarily in hospitalized patients and most likely complicates the course of critically ill [3]. Meanwhile, chronic kidney disease, which is also known as chronic kidney insufficiency, is a progressive disease as the presence of irreversible kidney damage. Patients suffer from this disease because of loss of quality of life [1,4].

Nowadays, people's paradigm has been shifting and the tendency to go back to nature becomes larger, particularly in Indonesia. Thus, the use of traditional medicine increased significantly. There are several herbs that are claimed having efficacy on renal failure state. One of them is breadfruit leaves (*Artocarpus altilis*). This plant has been used empirically to improve renal damage.

Basically, breadfruit leaves contain phenolic compounds, flavonoid, tannin, phenol, hydrocyanic acid, potassium, polyphenol, acetylcholine, and saponin. Flavonoid, quercetin, and arthoindocianin are compounds that pharmacologically active [5]. Several research on breadfruit leaves has been performed, such as the study on its antioxidant activity [6] and as antibacterial agent [7]. Breadfruit extracts and its metabolites from its organs - leaves, stem, fruit, and bark-contain numerous secondary metabolites which are biologically active and possess antibactertial activity, antitubercular, antiviral, antifungal, antiplatelet, anti-arthritis, inhibit tyrosnase, and sows cytotoxicity [8,9]. The fruit can be employed to treat the gastrointestinal disorder, and then the stem has been proven having anticancer effect against breast cancer cell T47D [10], reducing cholesterol level either in blood or tissues, and it shows anti-atherogenic activity [11]. In traditional use, breadfruit leaves are used for treating renal, liver, and heart disorders. However, there is no scientific proven on breadfruit leaves in the renal failure.

Using the same model as our previous study, renal failure model was performed by injecting gentamicin via intraperitoneal route and piroxicam orally [12]. There are several studies proving that gentamicin may disrupt renal function and increase values of biochemical parameters related to kidneys disorder [12-16]. The combination of gentamicin and piroxicam deteriorates renal function faster that of gentamicin alone. Following administration of piroxicam may keep damage of kidneys throughout the treatment period [12].

Thus, the objective of this research was to observe the influence of extract of breadfruit leaves in ameliorating kidneys function by using rat's model which are induced by gentamicin and piroxicam.

METHODS

Chemicals

All chemical reagents for phytochemical screening which are include: Toluen, chloroform, ammonia, Dragendorff reagent, Mayer reagent, hydrochloric acid, magnesium powder, amyl alcohol, gelatine, ferri chloride, Steasny reagent, acetic sodium, sodium hydroxide, acetic acid

anhydride, sulfuric acid; gentamicin injection (Indofarma); piroxicam (Indofarma); NaCl 0.9%; CMC Na; creatinine reagent kit (®Rajawali Nusindo), urea reagent kit (®Rajawali Nusindo), reagents that is necessary needed in histological study, formalin phosphate buffer 10%. All chemicals used meets pharmaceutical grade standard.

Apparatus

Grinding instrument, analytical balance, reflux apparatus, centrifuge instrument, microcentrifuge tube, oral gavage, syringe, rat restrainer, scissors, micropippette, UV-Vis spectrophotometer (®Beckman Coulter DU 720), photometer (®Robert Riele K. G. 5010 V5+), surgical apparatus, object glass, cover glass, electrical binocular microscope.

Animals

Male Wistar rats with approximately 2-3 months in age and 150-300 g in weight were used in this study. Rats were provided by Animal Laboratory of School Pharmacy of ITB and kept under usual management condition in this institution. All conducted methods have been approved by Ethics Comission ITB and documented with the code of 02/KEHP-ITB/03-2015.

Plant material

Breadfruit leaves (*A. altilis* [Parkinson] Fosberg) was purchased from Manoko, Lembang, West Java, Indonesia. This crude herbs was then determined in Herbarium Bandungense, School of Natural Science and Technology ITB, Indonesia.

Preparation of extract

Powdered crude of breadfruit leaves was extracted with ethanol 96% in reflux apparatus. The extraction was conducted three times and filtered through Whatman filter paper. It was followed by evaporation using rotary evaporator until the viscous extract was obtained. This extract was referred as an ethanolic extract of breadfruit leaves (EEBL). It was kept in refrigerator 4°C until it would be used for pharmacological studies.

Experimental design

All rats were randomly divided into five groups, including normal, positive control, group which was given by EEBL 50 mg/kg, EEBL 100 mg/kg, and EEBL 200 mg/kg. The extract was dispersed into CMC Na 0.5% solution.

All rats, except that belong to a normal group, were treated by gentamicin 100 mg/kg/day intraperitoneally and piroxicam suspension 3.6 mg/kg/day orally for 7 consecutive days to induce kidney failure [17]. The group which was served as a normal group was injected by normal saline and CMC Na 0.5% by a oral route. For positive control, after the induction of kidney failure, piroxicam was continuously given until the 4th week of the treatment period, and rats belong to this group received the vehicle (CMC Na 0.5%) via the oral route. Meanwhile, all treated groups received breadfruit extract once daily according to the doses of each group until the 4th week of therapy. Together with the administration of extract, all rats received piroxicam suspension once daily.

Serum creatinine and urea level were determined at the first time before conducting induction (T0) and measured again a week after induction (T1). Then, measurement on both parameters was performed every week began from the 1st of therapy (T2, T3, T4, T5).

Evaluation of kidneys function

To evaluate kidney function, measurement of serum urea and creatinine levels were conducted. Blood sampling was performed at the period: Before induction (T0), after induction (T1), and every week after induction (until the 4th week: T2-T5). Blood was collected through vena lateral in tail regions and then transferred into a microcentrifuge tube. Whole blood should be centrifuged for 10 minutes to obtain serum. Afterward, serum must be stored in the freezer −20°C until analyzing time.

Determination of serum creatinine and urea level

Serum creatinine level was measured by enzymatic reaction kit according to the kinetic method of Jaffe. The concentration was calculated from obtained absorbance at 546 nm through spectrophotometer instruments.

Serum urea level was determined by urease enzymatic kit ®Rajawali Nusindo Indonesia according to Berthelot reaction. The presence of water will hydrolyze urea in a sample, then by urease from the kit; it will produce ammonia and carbon dioxide. Ammonia will react with hypochlorite, thus will be catalyzed by nitroferricyanide to give dark blue/green solution. The intensity of this color is relevant to urea concentration in the sample which is detectable on the wavelength of 578 nm.

Histological studies

24 hrs on accomplishing the treatment, all rats were sacrificed by placing them on the particular chamber. Through certain pipes, CO_2 was allowed to flow into the chamber. After sacrifice, the kidneys from each rat were quickly isolated and then fixed into formalin buffer which was followed by paraffin embedded and were stained by hematoxyline eosin for histopathological studies. The study was carried out through light microscopical observation.

Statistical analysis

All parametric data were evaluated using one-way ANOVA, which was followed by LSD *post-hoc* analysis by SPSS IBM software version 22. The value of <0.05 was taken as a significant point.

RESULTS

Crude leaves and extract standardization

With regards to the herbs determination in Herbarium Bandungense, School of Natural Science and Technology ITB, the used herb is *A. altilis* (Parkinson) Fosberg. The results of characterization were shown in Table 1.

Effect of gentamicin and piroxicam administration for 7 days consicutevely on serum creatinine and urea level

Measurement of urea serum is shown on Table 4, meanwhile creatinine serum is depicted on Table 5. There was a noticeably increased after the

Table 1: Results of crude herbs characterization

Parameter	Result (%)
Ash content	18.97
Water extractable matter	6.80
Ethanolic extractable matter	3.30
Volatile content	13.00

Table 2: Extract standardization

Parameter	Result
Yield extract	9.48% (b/b)
Specific gravity	0.95 g/ml
Water extractable matter	1.11%
Ethanolic extractable matter	51.83%
Volatile content	5.80%

Table 3: Phytochemical screening

Secondary metabolite group	Result
Alkaloid	-
Flavonoid	+
Tannin	+
Phenol	+
Saponin	+
Quinon	+
Steroid/Triterpenoid	-

+: detected, -: Undetected

Table 4: Measurement of urea level during the experimental period

Group	Concentration of urea serum (mg/dl)					
	T0	T1	T2	T3	T4	T5
Normal	38.76±2.30	42.39±4.78	45.40±12.27	38.70±5.43	33.92±3.24	30.52±8.67
Control positive	35.97±3.25	141.03±11.77*	227.32±201.06*	69.13±34.86	68.64±34.01*	58.05±18.88*
EEBL 50 mg/kg	32.94±6.01	113.74±61.67*	201.68±128.99	80.41±25.54*	59.89±23.15	51.97±14.01*
EEBL 100 mg/kg	36.39±8.55	72.27±43.94	120.83±44.60	49.08±11.36	59.63±10.33	47.43±11.15
EEBL 200 mg/kg	28.79±4.42*	107.22±42.91	89.17±36.11	50.07±4.97	44.00±6.41	51.01±7.03

Values are expressed as mean±SD, *Significantly different from control group (p<0.05), EEBL: Ethanolic extract of breadfruit leaves, SD: Standard deviation

Table 5: Measurement of urea level during the experimental period

Group	Concentration of creatinine serum (mg/dl)					
	T0	T1	T2	T3	T4	T5
Normal	38.76±2.30	42.39±4.78	45.40±12.27	38.70±5.43	33.92±3.24	30.52±8.67
Control positive	35.97±3.25	141.03±11.77*	227.32±201.06*	69.13±34.86	68.64±34.01*	58.05±18.88*
EEBL 50 mg/kg	32.94±6.01	113.74±61.67*	201.68±128.99	80.41±25.54*	59.89±23.15	51.97±14.01*
EEBL 100 mg/kg	36.39±8.55	72.27±43.94	120.83±4.60	49.08±11.36	59.63±10.33	47.43±11.15
EEBL 200 mg/kg	28.79±4.42*	107.22±42.91	89.17±36.11	50.07±4.97	44.00±6.41	51.01±7.03

Values are expressed as mean±SD, *Significantly different from control group (p<0,05), EEBL: Ethanolic extract of breadfruit leaves, SD: Standard deviation

induction period, in terms of urea and creatinine concentration in rats serum (Tables 4 and 5, T1 parameter). Even though only positive control group and the group treated by extract with the dose of 50 mg/kg body weight (bw) which showed a significant difference from the normal group, urea, and creatinine serum in all induced rats elevated up to five folds than that of the normal.

Effect of breadfruit leaves extract on serum urea level

The concentration of urea serum was relatively stable in the normal group throughout the periods. However, in all induced group, the level of urea increased beginning from T1. The values became even higher in T2 period, except that of EEBL 100 mg/dl, in which it reduced to 89.17 mg/dl. In T3 and T4, despite the level of urea decreased in all groups, control positive showed significant disparity from the normal group. At the end of treatment, only rats in both control positive and EEBL 50 mg/dl remained to suffer from kidney damage.

Effect of breadfruit leaves extract on serum creatinine level

The results on creatinine serum measurement showed the same tendency with that of urea determination. After a week induction of gentamicin-piroxicam, the value of creatinine in serum increased as double to fourth as that of the normal group. The number continuously went up in week 2 (T2), except for EEBL 200 mg/kg group. However, there was a reduction in terms of creatinine serum level on weeks 3 and 4, respectively. In the 5th week, creatinine serum level on groups treated by EEBL with the dose of 100 and 200 mg/kg bw were the same with that of the normal group.

Effect of breadfruit leaves extract on kidneys: Histological study

Examination on renal histology displayed that in the normal group, glomerulus was physiologically normal without neither presence of segmentation nor atrophy. In contrast, glomerular segmentation and atrophy were distinctively seen in the positive control group. Rats treated by EEBL showed improvement in a dose-dependent manner. The highest dose ameliorated structure of kidneys most, microscopically, in which the anatomy was similar to that of the normal group (Fig. 1).

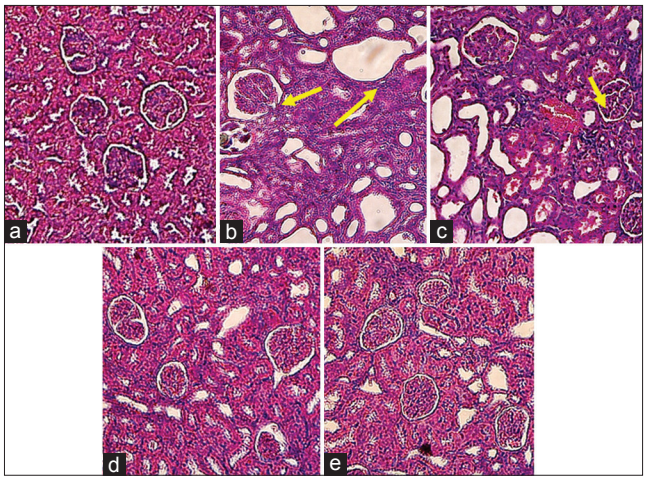

Fig. 1: Appearance of transversal plan of renal cortex, with ×100 optical zoom. (a) normal, (b) positive control, (c) ethanolic extract of breadfruit leaves (EEBL) 50 mg/kg, (d) EEBL 100 mg/kg, (e) EEBL 200 mg/kg. Arrow pointed at atrophic glomerular and renal segmentation

DISCUSSION

Extraction was conducted three times to increase the yield of extract. Liquid extract was then concentrated by rotary evaporator. Characterization and standardization are crucially needed to assure that quality of crude plants and extract meets the standard.

Gentamicin is widely used in inducing renal failure in experimental studies. Gentamicin belongs to aminoglycoside antibiotics that result in renal disorders due to its irreversible adverse effect. In addition, several studies have proven that toxicity of aminoglycosides including gentamicin is related in generating reactive oxygen species in renal mitochndria [14,18,19]. It increased renal malondyaldehide, nitric oxide, blood urea nitrogen, and creatinine levels but decreased several biochemical parameters including glutathione peroxidase, superoxide dismutase, catalase, and glutathione content [19]. Gentamicin is specifically accumulated in lisosomal proximal tubule which leads to lisosomal dysfunction and cause cell lysis in the tubule regions [20]. Thus, tubule will lose its capability to eliminate metabolic waste. In the long-term administration, it may cause renal failure.

Piroxicam which is classified in non-steroidal anti-inflammatory drugs (NSAIDs) group inhibits prostaglandine synthesis. Although it can be employed as analgesic, antipyretic, anti-inflammation, it shows untoward effects such as gastrointestinal disturbance and nephrotoxicity. The exact mechanism of NSAID-induced nephrotoxicity remains unclear, but most theories believed that it is closely related to the particular enzyme, called cyclooxygenase which converts arachidonic acid to prostaglandine. In the very beginning, inhibition

of prostaglandine synthesis may lead to a reduction of blood flow to kidneys that promote compensatory vasodilatory disequilibrium. In the following step, it deteriorates glomerular filtration rates [12]. However, such an adverse effect may occur in the presence of other nephrotoxic agents. So that, additional NSAID will potentiate renal damage in combination with aminoglycoside [12,17].

In general, administration of gentamicin and piroxicam together for 7 days cause an increased on creatinine and urea level, except the normal group. Levels of urea went up by two to five holds than that of normal rats. Such an elevation occurred because of renal damage; thus, urea that should be eliminated remained circulated in cardiovascular system.

In the end of the 2nd week, urea serum level still increased. It happens most likely because active compounds in extracts have not effectively eliminated urea and other toxic compound through the kidneys. However, the concentration of urea levels on rats treated by EEBL 200 mg/kg slowly decreased. The higher dose given to rats, the greater amount of compounds that potentially heal renal damage. Beginning on the 3rd week, both urea and creatinine serum levels dropped in all treated groups. This phenomena may prove that there was a homeostatic response to achieve normal state as nephrotoxic given, in which both parameters return to its normal value as time goes by. At the end of period, only rats treated by EBLL 200 mg/kg bw showed the similar values with that of normal group ($p>0.05$).

Pharmacological activities of breadfruit extract on renal failure are not scientifically proven. There was an improvement on rats motoric activities on multiple sclerosis model [21]. Other research stated that breadfruit leaves extract actively against pathogenic microbes such as *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans*, and *Enterococcus faecalis* [22]. It was believed that the activity related to phytochemical compounds such as steroids, phytoesterols, gum, resins, phenols, terpenoids, and flavonoid [22]. In addition, methanolic extract of breadfruit leaves had a protective effect against dietary cholesterol-induced hypercholesterolemia [11]. Additional studies conducted by Sikarwar [23] stated that flavonoids are responsible to the antioxidant activities of breadfruit leaves extract. In her publication, Riasari has identified flavonoid compounds as a major component in breadfruit leaves [24]. The ability of breadfruit leaves extract on improving renal failure state is very likely related to these findings.

According to histological studies, administration of EEBL with the dose of 200 mg/kg may contribute to prevention or repairment of damaged cells. In the control group, the degenerated and desquamated epithelias cells existed in the lumen of tubules [12,25,26]. Extracts which were given with lower dose showed structural improvement in kidney cells, even though the appearance was not as good as that of EEBL 200 mg/kg treated group. Breadfruit leaves extract play a role to amend creatinine and urea serum levels, as well as histopathological findings. There are possibilities of EEBL on rehabilitating kidney cells and optimizing remain existed cells.

CONCLUSION

Administration of EEBL with various doses seems to improve biochemical parameters related to kidneys function and the structure of kidney cells, with dose-dependent manner. EEBL with a dose of 200 mg/kg reveals the highest activities to ameliorate kidneys performance.

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