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RESEARCH ARTICLE

Acute Toxicity Test of Unripe Papaya (*Carica papaya* L.) Aqueous Extract (UPAE) on The Blood Urea and Creatinine Concentration

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Abstract

Unripe papaya aqueous extract (UPAE) widely used as lactation stimulator, antidiabetes, antibacterial, and anti-inflammatory. The utilization of papaya is not known for its safety yet, so it is necessary to research its toxicity. The purpose of this study was to investigate the acute toxicity of UPAE on renal function through measurement of blood urea and creatinine levels. This study was conducted in July 2017 in Laboratory of Medical Biology, Faculty of Medicine, Universitas Islam Bandung. This study used pure in vivo experimental design on 11 Swiss Webster mice using the dose of acute toxicity determination based on new recommended methods of 0; 50; 200; 400; 800; 1,000; 1,500; 2,000; 3,000; 4,000; and 5,000 mg/kgBW. After 24 hours, 1 mL blood drawn through the tail examined for blood urea and creatinine levels. The measurement of urea content using kinetic method point and creatinine level using modified Jaffe method. Provision of UPAE at doses of 0, 50, 200, 400, 800, and 1,000 mg/kgBW resulted on blood urea equal to 39, 35, 48, 49, 48, and 32 mg/dL respectively. Blood urea level 23, 22, 28, 34, and 35 mg/dL was obtained at 1,500 UPAE doses; 2,000; 3,000; 4,000; and 5,000 mg/kgBW dosages respectively. After 24 hours of UPAE administration, the creatinine level in various doses using new recommended method of (0–5,000 mg/kgBW) were 0.75, 0.54, 0.53, 0.50, 0.60, 0.54, 0.52, 0.55, 0.42, 0.51, and 0.40 mg/dL. In conclusion, UPAE do not cause acute toxicity on renal function through measurement of blood urea and creatinine levels.

Keywords: Acute toxicity, unripe papaya aqueous extract

Toksisitas Akut Ekstrak Air Buah Pepaya (*Carica papaya* L.) terhadap Kadar Ureum dan Kreatinin Darah

Abstrak

Ekstrak air buah pepaya muda (EABPM) digunakan secara empiris sebagai laktagogum, antidiabetes, antibakteri, dan antiinflamasi. Tingkat keamanannya belum banyak diketahui sehingga perlu dilakukan penelitian uji toksisitas akut. Tujuan penelitian ini adalah mengetahui toksisitas akut EABPM terhadap fungsi ginjal melalui pengukuran kadar ureum dan kreatinin plasma. Penelitian ini dilaksanakan pada bulan Juli 2018 di Laboratorium Biologi Medis, Fakultas Kedokteran, Universitas Islam Bandung. Penelitian ini menggunakan desain eksperimental murni *in vivo* terhadap 11 ekor mencit betina galur Swiss Webster dengan penentuan dosis sesuai dengan *new recommended method*: 0, 50, 200, 400, 800, 1.000, 1.500, 2.000, 3.000, 4.000, dan 5.000 mg/kgBB. Setelah 24 jam, diambil darah melalui ekor mencit sebanyak 1 mL untuk diperiksa kadar ureum dan kreatinin plasma. Pengukuran kadar ureum menggunakan *point kinetic method* dan kadar kreatinin menggunakan metode Jaffe yang dimodifikasi. Pemberian EABPM pada dosis 0, 50, 200, 400, 800, dan 1.000 mg/kgBB didapatkan kadar ureum plasma 39, 35, 48, 49, 48, dan 32 mg/dL secara berurutan. Kadar ureum plasma 23, 22, 28, 34, dan 35 mg/dL didapatkan pada pemberian dosis EABPM sebanyak 1.500, 2.000, 3.000, 4.000, dan 5.000 mg/kgBB. Kadar kreatinin plasma dalam berbagai dosis (0–5.000 mg/kgBB) adalah 0,75; 0,54; 0,53; 0,50; 0,60; 0,54; 0,52; 0,55; 0,42; 0,51; dan 0,40 mg/dL. Simpulan, EABPM tidak menimbulkan tanda toksisitas akut pada fungsi ginjal melalui pengukuran kadar ureum dan kreatinin plasma.

Kata kunci: Ekstrak air buah papaya muda, toksisitas akut

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Introduction

Indonesia is a country rich in natural materials, especially medicinal plants (herbs), with approximately 25,000 to 30,000 plant species found. The community uses a total of 7,000 plant species in Indonesia as medicine, and only 283 species registered with the Food and Drug Administration (BPOM).[1] The use of herbal medicine through the utilization of natural materials has become a tradition for generations. Research on medicinal plants has increased rapidly along with the growing use of medicinal plants in the treatment effort in the community, especially the traditional community.[2,3]

Medicinal plants are used empirically and are considered safe for consumption because they come from nature. However, the use of traditional medicines should have a scientific basis for their safety and effectiveness by identifying active substances. Ethno-pharmacology/ethno-pharmaceutical that performing pre-clinical tests of plant extracts on experimental animal models is essential.[4]

Papaya (*Carica papaya* L.), *Carica* clan *Caricaceae* is a plant that is widely studied today. Papaya has a wide range of medicinal benefits due to their abundant and active compounds such as papain enzymes, carotenoids, alkaloids, monoterpenoids, flavonoids, minerals, vitamins, glucosinolates, and carposides. Benefits of papaya in medicine include anticancer, antioxidant, antidiabetic, antifertility, anti-inflammatory, antihelminthic, antibacterial, antimalarial, anti-dengue, and wound healing.[5]

Active compounds in papaya can provide several benefits if used in the appropriate dosage. Examples of papaya active compounds are alkaloids, saponins, and flavonoids which have antibacterial, antioxidant, anti-inflammatory effects, as well as lactation stimulant (lactagogue). However, if the utilization of the papaya is not suitable for its use, it can have adverse effects. Such adverse effects will result in functional impairment of organs and cells.[6] Thus, toxicity and safety testing of herbal products and natural ingredients is an important thing to do. The World Health Organization (WHO) puts the issue of traditional drug safety into one of the essential steps in Traditional Drug Development Strategy of 2014–2023.[7]

Toxicity can cause damage to several organs of the body. Toxicity tests are needed to assess the safety of a drug, as well as substances recognized as supplements or foods. This test can protect the public against the adverse effects of a drug preparation. One such toxicity test is an acute toxicity test, a test to identify undesirable effects at the first 24-hour interval after administration of one/several substances. One of the initial screening assessments of acute toxicity of test preparation is the calculation of lethal dose 50 (LD50).[8,9]

Based on research that has been done using Karber method it is known that unripe papaya aqueous extract (UPAE) LD50 (*Carica papaya* L.) acute toxicity test is 2,520 mg/kgBW.[10] In research conducted by Nadiyah et al.[11] LD50 from papaya fruit extract (*Carica papaya* L.) found to be >5,000 mg/kgBW. Acute toxicity of unripe papaya fruit juice extract has also performed on erythrocyte morphology. This study states that UPAE does not result in erythrocyte morphological changes in microscopic examination of a peripheral blood smear.[6]

The kidneys are vital organs that are essential for the body and perform various functions. They include the excretion function of metabolism and foreign substances that the body does not need, the water balance and electrolyte arrangement. Also involves in the regulation of the osmolarity of the body fluids and the electrolyte concentration, the acid-base balance arrangement, the arterial pressure, hormone secretion and gluconeogenesis.[12] Measurement of blood urea and creatinine levels is one of the markers on renal function evaluation. The increase in serum levels of the enzyme indicates the occurrence of renal injury.[13]

The purpose of this study was to investigate the acute toxicity of UPAE on renal function through measurement of blood urea and creatinine levels.

Methods

This study used purely in vivo experimental design on eleven Swiss Webster mice aged 6–8 weeks weighing 25–30 grams. Research subjects were divided into four 77.4 cm^2 by 12.7 cm plastic tubs with woven wire cover. The room set to a temperature of about 19–25°C, freed from predator and sanitation maintained. Mice went to adaptation period of seven days.

The research material is 2.5–3 months old unpeeled papaya fruit (including seed) from plantation in Leles sub-district, Garut regency,

West Java. The selected papaya is unripe fruit with dark green skin, smooth, no defect, white flesh and seed, picked from healthy plants. This experimental laboratory study conducted at the Laboratory of Medical Biology, Faculty of Medicine, Universitas Islam Bandung (Unisba) in July 2018. The proposed (new) recommended method selected for use in this toxicity test study were 0; 50; 200; 400; 800; 1,000; 1,500; 2,000; 3,000; 4,000; and 5,000 mg/kgBW. After 24 hours, 1 mL blood was collected through the tail to check blood urea and creatinine levels. At the end of the study, research subjects sacrificed through decapitation. This study have obtained ethical clearance from Health Research Ethics Committee, Faculty of Medicine, Unisba number: 095/Komite Etik FK/III/2017.

Results

Phytochemical screening results of UPAE is obtained from secondary metabolite in the form of saponin, alkaloid, flavonoid, triterpenoid, quinone, and tannin.

In this study, blood urea content of the research subjects without UPAE (control) was 39 mg/dL. Provision of UPAE at dose 50, 200, 400, 800, and 1,000 mg/kgBW showed the result of blood urea of 35, 48, 49, 48, and 32 mg/dL respectively. The plasma urea content with value 23, 22, 28, 34, and 35 mg/dL was obtained on the subject of the study by UPAE dosages of 1,500; 2,000; 3,000; 4,000; and 5,000 mg/kgBW.

Plasma creatinine level after 24 hours of UPAE in various doses using new recommended method (0–5,000 mg/kgBW) were 0.75, 0.54, 0.53, 0.50, 0.60, 0.54, 0.52, 0.55, 0.42, 0.51, and 0.40 mg/dL.

Table 1 Phytochemical Screening of Unripe Papaya Aqueous Extract

Secondary Metabolite	Reaction	Result
Saponin	Sample + HCl (shaken)	+
Alkaloid	Sample + Dragendorff reagent + $CHCl_3$+HCl	+
Flavonoid	Sample + Mg + HCl + amyl alcohol	+
Terpenoid	Sample + ether + H_2SO4 + acetic acid glacial	+
Quinone	Sample + NaOH	+
Tannin	Sample + Stiasny reagent	+

Table 2 Blood Urea and Creatinine Measurement

Dosages (mg/kgBW)	Blood Urea (mg/dL)	Creatinine (mg/dL)
0	39	0.75
50	35	0.54
200	48	0.53
400	49	0.50
800	48	0.60
1,000	32	0.54
1,500	23	0.52
2,000	22	0.55
3,000	28	0.42
4,000	34	0.51
5,000	35	0.40

Discussion

The kidney is a vital organ that keeps the body homeostatically by regulating water and electrolyte balance, regulating acid-base balance, and regulating the osmolarity of body fluids and electrolytes. The kidneys selectively excrete solutes and water and throw out the rest of the metabolism. Kidney damage can be caused by toxins or medications that damage nephron epithelial cells.[15]

The measurement of the biochemical parameters of urea and creatinine levels was performed as an initial stage of evaluation of renal function that was used as one of the benchmarks of acute organ damage. Ureum and creatinine are also indicators of glomerular filtration rate (GFR) which is a marker of kidney function.[13] Impaired kidney function leads to decreased renal filtration rate, accompanied by accumulation of metabolic (urea and creatinine) residues in the blood so that the levels of these two substances in this blood can be used as an indicator of the degree of kidney health.[16]

Urea is a major metabolite derived from protein intake as well as breakdown of tissue proteins while creatinine is a product of muscle creatin catabolism. The range of urea values is

quite wide due to normal variation due to protein intake, endogenous protein catabolism, hydration state, hepatic urea synthesis, and renal urea excretion.[17,18] Increased urea levels can be caused by an increase in the catabolism of tissue proteins accompanied by negative nitrogen balance, an excessive protein-breaking process occurs in cases of leukemia in which leukocyte protein leaks, presence of urea-erythrocyte disorder, due to prerenal, renal or postrenal disorder or due to high protein consumption.[13]

Creatinine is a type of amino acid that is a waste product in the blood and excreted through the kidneys into the urine. Generally, creatinine stores in muscles as energy reserves in the form of a creatinine-phosphate source of adenosine triphosphate (ATP). Another place that produces creatinine is in the liver, pancreas, and kidneys. High creatinine levels are thought to be due to severe muscle activity (hard exercise) or due to impaired kidney discharge systems. Creatinine levels are relatively stable because protein does not influence them from the diet.[18]

Measurement of urea and creatinine levels in this study showed different values when compared with controls. However, this is not an indicator of renal impairment because the parameters of renal impairment are indicated by an increase in blood creatinine levels doubled from the normal range and occurring for more than two weeks.[19,20] This may be due to a decline in kidney function as part of the physiological dynamics. The fluctuations occurring in the study subjects did not indicate renal impairment because they are not accompanied by a doubling of urea levels in the range of control values (average) and not followed by a parallel increase in creatinine.[19,21]

Further research is needed on UPAE toxicity through sub-chronic and chronic toxicity tests to complement the safety data in the form of morphological, functional, and life-long changes in the subject.

Conclusion

Provision of unripe papaya fruit extract by using a dose of acute toxicity determination based on new recommended method did not affect renal function.

Conflict of Interest

All authors state whether there was not a conflict of interest in this article.

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