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Kidney tubular injury of rats caused by unripe green betel nuts (*Areca catechu*)

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ABSTRACT

Background: Unripe green betel nuts (*Areca catechu*) are usually consumed as herbal medicines by some inhabitants in several regions of Indonesia. It is considered that the nuts contain Arecoline hydrobromide that can cause damages in a kidney tubule, although it needs further studies about effects of the nuts.
Objective: This study aims to determine the effects of the unripe green betel nuts on kidney tissues of rats in a long-term treatment.
Methods: Twenty rats used in this study were Sprague-Dawley strain, male, 2-3 months old, and 150-200 gram of weight. They were randomly divided into four groups. Group I was control group, given distilled water once a day; group II, III, and IV were served by juice of the unripe green betel nuts with a dose of 250 mg/kgBW, 1,000 mg/kgBW, and 10,000 mg/kgBW, respectively. Histopathology examination by staining of Haematoxylin Eosin was conducted by an anatomic pathologist using blind methods including glomerulus, tubulus, and interstitial tissues.
Result: The tested kidney tissues showed mild interstitial congestion, tubular cast, and tubular degeneration in groups that received unripe green betel nuts. There were no glomerulus abnormality and interstitial inflammation. The highest percentage of rats suffering injury in their kidney was the group IV, with p value <0.05 when compared to the control group.
Conclusion: Long-term consumption of unripe green betel nuts could cause kidney tubular injury.

Latar Belakang: *Biji pinang muda dimanfaatkan oleh sebagian masyarakat di Indonesia sebagai minuman herbal tradisional. Arekolin hydrobromida menyebabkan kerusakan ginjal. Biji pinang mengandung arekolin dan senyawa lainnya. Konsumsi biji pinang diduga berkaitan dengan penyakit ginjal kronik. Efek biji pinang terhadap organ ginjal belum diketahui secara pasti.*
Tujuan Penelitian: *Mengetahui efek pemberian biji pinang muda jangka panjang terhadap jaringan ginjal tikus.*
Metode: *Dua puluh ekor tikus galur Sprague Dawley, jantan, berusia 2-3 bulan, berat badan 150-200 gram dibagi secara random dalam 4 kelompok. Kelompok I sebagai kelompok kontrol diberikan akuades, kelompok II, III dan IV diberikan jus pinang masing-masing dosis 250 mg/kgBB, 1.000 mg/kgBB dan 10.000 mg/kgBB sekali sehari selama 45 hari. Pemeriksaan histopatologi dengan pewarnaan Hematoksilin Eosin dilakukan oleh ahli patologi anatomi secara blind meliputi glomerulus, tubulus dan jaringan interstitial.*
Hasil: *Jaringan ginjal pada kelompok perlakuan dengan biji pinang ditemukan kongesti interstitial ringan, tubular cast dan degenerasi tubular. Abnormalitas glomerulus dan inflamasi interstitial tidak ditemukan. Persentase tertinggi tikus dengan kerusakan ginjal terdapat pada kelompok pinang dosis 10.000 mg/kgBB dengan nilai p <0,05 ketika dibandingkan dengan kelompok kontrol.*
Kesimpulan: *Konsumsi biji pinang jangka panjang dapat menyebabkan kerusakan tubulus ginjal.*

INTRODUCTION

Betel nuts (*Areca catechu*) are one of the fruits consumed widely throughout the world.[1,2] In South Asia, the nut is used as a mouth freshener after meals, taste enhancer, laxative, medication to parasitic intestinal infections, impotence, and gynaecological problems.[3] In Sumatra (Indonesia), the nut is consumed as an herbal medicine for stamina booster or a hypoglycemic agent, with a dose of 250 to 1,000 mg/kgBW. The nut has a median lethal dose about >10 gr/kgBW.[4]

Negative effects of betel nut consumption on health have been reported.[5] There were several cases of acute toxicity of the nuts in men that could be recovered within 24 hours after exposure.[6] However, effects of high doses or long-term exposures of the nuts needs further studies. In some cases, chewing the betel nut is considered that it relates to chronic kidney diseases in men. Several retrospective studies reported that betel nut chewers, especially men, have higher prevalence of chronic kidney disease with proteinuria manifestation.[7–9]

The betel nuts contain arecoline, a major alkaloid of betel nuts.[10] Based on in vitro and in vivo studies, arecoline is known as cytotoxic and genotoxic agents through oxidative stress-dependent mechanisms.[11,12] Treatment of arecoline hydrobromide in animal models could cause kidney damages. A histopathological study on rats' kidney after 14 days of treatment with arecoline hydrobromide resulted granular degeneration, focal necrosis, and moderate congestion.[13]

The betel nuts also have other phytochemical constituents beside the alkaloid, such as polyphenols. A treatment with the betel nuts may have different histopathological changes than the arecoline hydrobromide. Effects of a long-term treatment with the betel nuts in histopathology of the kidney need further studies. This study aims to examine types of kidney injuries by a histopathological assessment after a long-term treatment with the betel nuts in rats.

METHODS

Preparation of unripe green betel nuts

The unripe green betel nuts were collected from a plantation in Jambi. Specimen authentication was determined by Faculty of Biotechnology and Engineering Laboratory of Sciences and Technology, Jambi University. Stock solution of the nuts was freshly made every day during this study; 60 g of betel nuts was crushed into refined pieces and added with 60 ml of aquadest.

Design of study

The design of this study was approved by Ethics Committee of Faculty of Medicine and Health Sciences, Universitas Jambi Number 634/UN21.6/LT/2018. A number of 20 adult male Sprague Dawley rats were examined and randomly divided into four groups. The first group (I) was the control group receiving 1 ml of distilled water. The second (II), third (III) and fourth (IV) groups were served by betel nut stock solution with a volume equal to a dose of 250 mg/kgBW; 1,000 mg/kgBW and 10,000 mg/kgBW, respectively. The treatment to the groups was held for 45 days. All rats were housed in plastic cages by room temperature of 25°C and 50-80% humidity and with 12-hour cycle variations between light and dark, and they also were served by a standard diet and water ad libitum.

Histopathology

All kidney tissues collected during necropsy were fixed in 10% formalin. Following the fixation, they were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Sections of the tissues were stained with haematoxylin and eosin. The tissues were observed by anatomical pathologist by using a light microscope. This observation included glomerulus, interstitial congestion, interstitial inflammation, tubular cast and tubular degeneration. The changes in the observation were scored as follows: (0) showing no changes; (1) mild, less than 30% changes; (2) moderate, 30%-50% changes; (3) severe, more than 50% changes.[14]

Statistics

The data were presented in percentages.

The data were analysed by using Kruskal Wallis and then by Mann Whitney U test to compare mean values of histopatological scores in each parameter between the groups. A mean change was considered significant when p <0.05.

RESULTS

Histopathology assessment of the kidney tissues could be seen in Table 1 below. There were several changes in the kidney (kidney tubule) of the rats given unripe green betel nuts, such as interstitial congestion, tubular cast, and tubular degeneration. There was no abnormality of glomerulus and interstitial inflammation in the kidney of the rats given unripe green betel nuts. Based on Kruskal Wallis Test, there were significant results among all the groups for interstitial congestion, tubular cast, and tubular degeneration (p<0.05).

Table 1. Histopathology assessment of kidney in all groups of the rats

Parameters	Number and percentage of rats in each groups				p value
	I	II	III	IV	
Glomerulus					
No changes	5 (100%)	5 (100%)	5 (100%)	5 (100%)	
Mild	0	0	0	0	1.000
Moderate	0	0	0	0	
Severe	0	0	0	0	
Interstitial congestion					
No changes	5 (100%)	4 (80%)	2 (40%)	0	
Mild	0	1 (20%)	3 (60%)	5 (100%)	0.010*
Moderate	0	0	0	0	
Severe	0	0	0	0	
Interstitial inflammation					
No changes	5 (100%)	5 (100%)	5 (100%)	5 (100%)	
Mild	0	0	0	0	1.000
Moderate	0	0	0	0	
Severe	0	0	0	0	
Tubular cast					
No changes	5 (100%)	5 (100%)	5 (100%)	2 (40%)	
Mild	0	0	0	3 (60%)	0.018*
Moderate	0	0	0	0	
Severe	0	0	0	0	
Tubular degeneration					
No changes	5 (100%)	2 (40%)	4 (80%)	0	
Mild	0	3 (60%)	1 (20%)	5 (100%)	0.010*
Moderate	0	0	0	0	
Severe	0	0	0	0	

Statistic test by using Kruskal Wallis Test by comparing the mean of histopathological score.
*Significance at p-value <0.05

Based on the results of Mann Whitney test (Table 2 below), there were no significant differences between the group of rats with 250 mg/kgBW of the nuts, the control group and

the group rats with 1,000 mg/kgBW of the nuts. However, there were significant differences between the groups of with 10,000 mg/kgBW (IV) and the control group (I) for interstitial congestion and tubular degeneration. Among the groups receiving the betel nuts, there were no dose-dependence patterns since no consistent significance was found.

Table 2. Results of Mann Whitney test

Parameters	Groups					
	I and II	I and III	I and IV	II and III	II and IV	III and IV
Interstitial congestion	0.317	0.050	0.003*	0.221	0.014*	0.134
Tubular cast	1.000	1.000	0.050	1.000	0.050	0.050
Tubular degeneration	0.050	0.317	0.003*	0.221	0.134	0.014*

Statistic test by using Mann Whitney Test. * significance at p-value <0.05

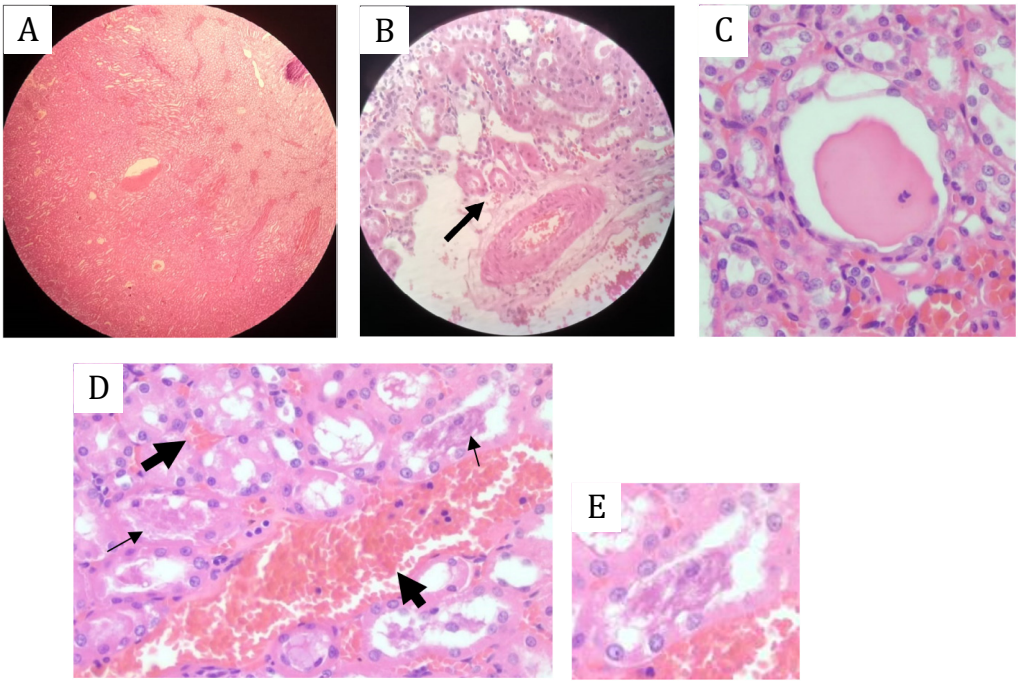

Figure 1. Histopathology of kidney tissues in groups treated with unripe green betel nuts. (A) Kidney tissues at 40-x magnification; (B) Tubular degeneration (arrow, 100-x magnification); (C) Hyaline cast (arrow, 400-x magnification), and it had smooth texture; (D) Granular cast (thin arrow, Granular cast had textured appearance) and interstitial congestion (bold arrow, red stain due to a presence of red blood cell, 400-x magnification); (E) Zoom of granular cast.

DISCUSSION

Several factors can make the kidney vulnerable to toxic injuries due to herbal medicines or drugs. There are several different types of mechanisms for herbal-induced or drug-induced nephrotoxicity, including changes in glomerular hemodynamic, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. NSAIDs have been shown to induce nephrotoxicity in the glomerulus. However, aminoglycoside, amphotericin B, adefovir, and cisplatin could cause tubular cell toxicity.[15]

Mechanism of nephrotoxicity of herbal agents can be classified into direct nephrotoxic, electrolyte abnormality, stone formation (oxalate stone) predisposition, diuretics actor, and heavy-metal substances or drug interaction.[16,17] The

toxins may directly injure the tubule, at the site of toxin transport or concentration, or by inducing kidney ischemia, hemoglobinuria, or myoglobinuria.[16] Based on the histopathology result of this study, the unripe green betel nuts might directly injure the tubule.

Histopathological assessment in this study found that unripe green betel nuts could cause interstitial congestion, tubular cast, and tubular degeneration with mild severity. These results were similar to the previous study about arecoline hydrobromide toxicity.[13] Based on a study examining arecoline toxicity in rats after a 14-day repeating oral administration with doses of 100, 200, and 1000 mg/kg, the arecoline hydrobromide caused moderate to severe tubular degeneration, focal necrosis, and congestion.[13] The betel nuts contained arecoline about 7.5 mg/g.[10,18] In this study, the whole compounds contained in the betel nuts were used without extraction, so an interaction between arecoline and other phytochemical compounds might occur.

Tubular, interstitial, and vascular injuries often accompany glomerular diseases. Morphological changes observed in the tubular, interstitial, and vascular compartments are nonspecific and also can be recognized in kidney diseases that do not have glomerular lessions as a component.[19] In this study, glomerular lessions were not found.

Proteinuria occurs secondarily to increase permeability of glomerular basement membranes or kidney tubules to serum proteins.[20] Tamm-Horsfall protein (THP) is a matrix of all casts. It is exclusively produced by kidney tubular cells of the distal loop of Henle. Casts consisting of only Tamm-Horsfall protein without presence of other cells are called hyaline casts which have smooth textures. Meanwhile, granular casts are generally results of a direct aggregation of serum proteins, other substances, or cell degeneration, such as red blood cells, white blood cells, or kidney tubular epithelial cells into a matrix of Tamm-Horsfall mucoprotein. Presence of the granular casts suggests stasis in the nephron. The Hyaline casts are normally seen in healthy animals but in low numbers, whereas granular casts with low numbers can indicate kidney injury.[21] Abnormal numbers of the casts are related to tubulointerstitial diseases.[22]

To find dose-dependent patterns of toxicity in the betel nuts in the kidney, Mann Whitney test was applied. Based on the results of Mann Whitney test (Table 2), there were significant differences of interstitial congestion and tubular degeneration between groups of rats with betel nuts of 10,000 mg/kgBW (IV) and the control group (I). However, there were no significant differences among the groups receiving the betel nuts. Therefore, it was supposed that there were no dose-dependence patterns of toxicity in the kidney caused by the nuts since consistent significance was not found, although a higher dose of betel nut tended to cause a higher prevalence of kidney damages.

The betel nuts were excreted through the kidney in the forms of 0.3 - 0.4% arecoline forms and the rest were in the other forms.[23] Several studies showed that arecoline could induce imparable DNA damages, repress DNA repair, and inhibit p53.[24,25] However, effects of arecolines' metabolites on the kidney need further observation.

CONCLUSION

This study found that unripe green betel nuts in a long-term treatment could cause kidney tubular damages in the rats, especially types of nephrotoxic. Whether arecoline or its metabolites are responsible for nephrotoxic effects of the nut need further studies. Thus, a further study in chronic toxicity of the betel nuts is recommended.

CONFLICT OF INTEREST

None declare.

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