

# EFFECTS OF COCONUT WATER (COCOS NUCIFERA SP.) ADMINISTRATION AS PREVENTION OF UROLITHIASIS IN CALCIUM OXALATE INDUCED - WHITE RAT WISTAR STRAINS

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## ABSTRACT

**Objective:** To analyze the effect of coconut water administration on the occurrence of urolithiasis in calcium oxalate-induced Wistar rats. **Material & Methods:** Laboratory experimental study with post-test only control group design, using experimental model of male *Rattus norvegicus*-Wistar strains. The treatment given is ad libitum administration coconut water to observe its effect on renal histopathological changes, microscopic analysis, and renal function markers. Urolithiasis induction uses exposure of 0.75% ethylene glycol (EG), and 2% Ammonium chloride (AC) in drinking water. **Results:** Descriptive analysis showed the tendency of decreasing in mean of renal function markers, and histopathological nephron damage in the treatment group versus control group: BUN (11.22 versus 14.29); Creatinine Serum (0.5308 versus 0.5714); degree of nephron damage (5.22 versus 10.36). In microscopic analysis, there were calcium deposits in the kidney tubules in the control group, which were not found in the treatment group. Based on the ANOVA test, obtained significant difference in BUN levels with  $p$  value=0.023 ( $p<0.05$ ), and the degree of nephron damage between study groups with  $p$  value= 0.000 ( $p<0.05$ ). There was no significant difference in serum creatinine with  $p$  value= 0.23 ( $p>0.05$ ). **Conclusion:** Coconut water is potential modality in inhibiting the deposition of calcium oxalate crystals, and protecting from impaired kidney function against urolithiasis in animal-model of calcium oxalate-induced Wistar rats.

**Keywords:** Calcium oxalate, coconut water, urolithiasis.

## ABSTRAK

**Tujuan:** Menganalisis efek administrasi air kelapa terhadap terjadinya urolithiasis pada tikus wistar yang diinduksi kalsium oksalat. **Bahan & Cara:** Penelitian eksperimental laboratorium post-test only control group design, menggunakan hewan coba *Rattus norvegicus* jantan strain Wistar. Perlakuan yang diberikan berupa administrasi air kelapa ad libitum untuk melihat pengaruhnya terhadap perubahan histopatologis ginjal, analisis mikroskopis tubulus, dan marker fungsi ginjal. Induksi urolithiasis menggunakan paparan 0.75% etilena glikol (EG), dan Amonium chloride (AC) 2% dalam air minum. **Hasil:** Analisis deskriptif menunjukkan kecenderungan penurunan rerata dari marker fungsi ginjal, dan kerusakan nefron secara histopatologis pada kelompok perlakuan versus kontrol positif: BUN (11.22 versus 14.29); Serum Kreatinin (0.5308 versus 0.5714); derajat kerusakan nefron (5.22 versus 10.36). Pada analisis mikroskopis, terdapat endapan kalsium didalam tubulus ginjal pada kelompok kontrol, yang tidak didapatkan pada kelompok perlakuan. Berdasarkan hasil uji ANOVA diperoleh perbedaan signifikan kadar BUN dengan nilai  $p=0.023$  ( $p<0.05$ ), dan derajat kerusakan nefron antar kelompok penelitian dengan nilai  $p=0.000$  ( $p<0.05$ ). Tidak terdapat perbedaan signifikan pada serum kreatinin kelompok perlakuan, dengan hasil uji ANOVA dengan nilai  $p= 0.23$  ( $p>0.05$ ). **Simpulan:** Air kelapa merupakan modalitas potensial dalam menghambat pengendapan kristal kalsium di jaringan ginjal serta mengurangi dan melindungi dari gangguan fungsi ginjal terhadap kejadian urolithiasis pada model hewan coba tikus putih strain Wistar yang diinduksi kalsium oksalat

**Kata Kunci:** Air kelapa, *Cocos nucifera*, kalsium oksalat, pencegahan, urolithiasis.

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## INTRODUCTION

Urolithiasis is a non-communicable disease that causes major problems in public health. More

than 1 million people come to primary health care centers and around 300.000 people go to the urology clinic every year because of urolithiasis. The prevalence of nephrolithiasis occurs in men which

are around 10% and 5% in women.<sup>1</sup> About more than 2 billion dollars is spent every year to treat this disease.<sup>2</sup> The number of nephrolithiasis in Indonesia based on data collected from hospitals throughout Indonesia was 37.636 new cases, with visits of 58.059 people, while the number of patients treated was 19.018 people, with the number of deaths amounting to 378 people or 1.98% of all the number of patients treated.<sup>3</sup> The results of the Indonesia Basic Health Research (Riskesdas) in 2013 showed the prevalence of nephrolithiasis in Indonesia based on the category that was once diagnosed, namely 0.6% of non-communicable diseases (PTM) and is the second-highest ranked disease in the urology section after BPH (Benign Prostate Hyperplasia).<sup>4</sup>

Nephrolithiasis is a common occurrence that affects up to 10%-15% of the human population. The incidence of kidney stones is increasing in the industrial world due to an increase in living standards, race, ethnicity, and geographical area. The study by Trinchieri et al. report recurrence rates of 50-75% after 10 and 20 years, respectively. Kidney stones are composed of CaOx, either alone or mixed with calcium phosphate until now the most common stones accounted for more than 80% of them. Previous studies have shown that tubular cell injury facilitates the formation of calcium oxalate crystals (CaOx) and deposition in renal tubules. Likewise, various authors show that calcium oxalate crystals increase lipid peroxidation even further causing kidney epithelial injury.<sup>5</sup>

Human kidney stones are the crystalline group of crystals and organic matrix. CaOx crystals are the main component reaching 80% of stones worldwide. Cracked rock surfaces reveal crystals arranged in concentric lamination and radial striation. Organic matrices permeate the entire stone and contain lipids, protein, and carbohydrates.<sup>6</sup> The formation of calcium stones is associated with various disorders, including renal tubular acidosis, hypercalcemia, hyperoxaluria, hypocitraturia, hypomagnesuria, and hyperuricuria.<sup>7</sup> Hyperoxaluria is caused by excess production or excessive absorption of oxalate by the intestine. Primary hyperoxaluria, where oxalate is overproduced because of interference in the oxalate biosynthetic pathway, is rare. Idiopathic CaOx stone formers are only mild hyperoxaluric, but chemically, CaOx crystals will form more easily with a slight excess of oxalate compared to excess calcium. About 60% of the stone formers are hypercalciuric, 30% hypocitraturic, and 10% hypomagnesuric.<sup>8</sup>

Although in recent years the development of kidney stone management with modern techniques such as Extracorporeal Shock Wave Lithotripsy (ESWL) and Percutaneous nephrolithotomy have revolutionized surgical management in kidney stones, but in the medical approach there has not been much innovation related to urolithiasis. Many medicinal plants have been used for centuries to treat urinary tract stones, although the mechanism of action was not well explained through systematic studies and pharmacological tests.<sup>9</sup>

Coconut water (*Cocos nucifera* L.) or liquid endosperm from coconuts, which contains sugar, vitamins, minerals, protein, free amino acids, and growth supporting factors. Coconut water is also a natural isotonic and is recognized in the tropics with many health benefits, where areas with habitual consumption of coconut water are found to have a low incidence of urolithiasis.<sup>10</sup> Providing young coconut water was also found to reduce the amount of crystals in the urine.<sup>11</sup> Researchers who conducted studies on populations in India, also found that coconut water could reduce the formation of urine crystals in experimental animals. However, so far no systematic research has been reported on the antiurolithiatic properties of coconut water in varieties and environmental conditions in Indonesia. This study was designed to evaluate the effectiveness of coconut water (*Cocos nucifera*) in animal models of urolithiasis.<sup>10</sup>

## OBJECTIVE

To find out the effect of giving coconut water (*Cocos nucifera* sp) as a protective effect of urolithiasis in calcium oxalate-induced Wistar rats.

## MATERIAL & METHODS

This type of research is experimental, using male white rat (*Rattus norvegicus*) Wistar strain. The treatment is given to subjects in the form of giving coconut water to see its effect on renal histopathological changes, microscopic analysis of tubules, and markers of kidney function.

The design of this study was an experimental laboratory with post-test only control group design, with the evaluation of the histopathological picture of the kidney which was carried out after the animals were treated. The grouping of experimental animals was carried out by randomization, with the repetition of 6 experimental

animals in each group and there was a control group as a comparison (positive control and negative control).<sup>12</sup>

Samples of male white rats (*Rattus norvegicus*) strain of Wistar, newly obtained from the Animal Laboratory of the Faculty of Veterinary Medicine, Airlangga University, Surabaya, began with an adaptation process in the cage/research environment for 1 week with a cycle of 12 hours of light, 12 hours of dark. The control group in this study included a negative control group (KN), who were treated with mineral water consumption as a placebo, then at week 2 this group would immediately take blood samples, and laparotomy to take kidney organs. The positive control group (KP), which was given the treatment of drinking mineral water consumption + 0.75% ethylene glycol + 2% ammonium chloride. Then there was the treatment group (P), namely the group that was given the treatment of drinking mineral water consumption + 0.75% ethylene glycol + 2% ammonium chloride at the end of the second week of the three groups then blood samples were taken, and laparotomy to take kidney organs, continued by making and examining microscopic preparations with paraffin method and hematoxylin-eosin staining and fixed with 10% formalin.

Renal function marker data, microscopic analysis of crystals in renal tubules and renal histopathology will then be tested for normality in order to determine whether the data is normal or not and will also be tested for variance to find the same data variant or not. The type of Hypothesis test that will be used in this study to determine the differences in the control group and the treatment group is determined based on the results of the normality test and data variance. If the data distribution is normal and the data variance is the same, then the One Way Anova hypothesis test is used.

The hypothesis is determined based on the significance value obtained. If the significance value  $<0.05$ , then the next step is to do a multiple comparison test or Post Hoc Test by Tukey, which is to find out in more detail the pairs of treatment groups that are significantly different and those that

are not significantly different.

If it does not meet the Anova test requirements, the following statistical test steps are performed<sup>13</sup>; (1) Efforts are made to transform the data so that the distribution becomes normal and the variants become the same; (2) If the variables resulting from data transformation are not normally distributed or the variants remain unequal, then the alternative is chosen by the Kruskal-Wallis test; (3) If the Kruskal-Wallis test produces a p-value  $<0.05$ , then the Mann-Whitney test is continued.

If there are differences, then proceed with the next statistical test to find out different data pairs (to see differences from each group). This test uses Mann Whitney as a further Kruskal Wallis test.

This study was significant if the p-value  $<0.05$ .<sup>13</sup> All data processing techniques were computerized analyzed using statistical product and service solution 20 for windows (SPSS 20).

## RESULTS

Rats were randomized using the simple randomized sampling method. Randomization of research samples aims to reduce research bias. To assess the normality distribution of the data, the Shapiro-Wilk analysis was used, because the number of samples was  $<50$ .

In all study samples, body weight (BB) of experimental animals had a mean that was not much different, with a mean of the negative control (202.22 g + 6.667), positive control (200.00 g + 8.165), and the treatment group (201.11 + 7.817). From the results of the normality test output with Shapiro Wilk, it is known that the entire group has a p-value  $>0.05$  which means the data is normally distributed. Further analysis was performed to assess differences in mean body weight of rats between groups using the One-way ANOVA parametric test. Based on the ANOVA test results obtained that the bodyweight variable with a value of Sig  $>0.05$  means that there was no significant bodyweight difference between treatment groups. From this analysis, it could be concluded that the randomization of research subjects has been successfully carried out.

**Table 1.** Table of rats weight distribution.

Group	Mean $\pm$ SD	P-value Shapiro-Wilk	P-value ANOVA
KN (Negative control)	202.22 $\pm$ 6.667	0.280	
KP (Positive control)	200.00 $\pm$ 8.165	0.144	0.842
P (Treatment)	201.11 $\pm$ 7.817	0.055	

From the results of the homogeneity test output, it was known that all groups have  $p\text{-value}=0.940$  ( $p\text{-value}>0.05$ ) which means homogeneous data. Descriptive analysis showed the average BUN in the negative control group was 9.89, the average BUN in the positive control group was 14.29, and the average BUN in the treatment group was 11.22. Thus it could be concluded descriptively that the highest average BUN was the positive control group given induction of urolithiasis without intervention.

In this study, the normality test was used by the Shapiro-Wilk analysis, because the number of samples was size  $<50$ . The results were normally distributed data ( $p>0.05$ ). From the results of the homogeneity test output, it is known that all groups had  $p\text{ value}= 0.415$  ( $p\text{-value} >0.05$ ) which means homogeneous data. Average data between groups were further analyzed to determine the difference in mean BUN levels between treatment groups with ANOVA. Based on the ANOVA test results obtained that the BUN variable with a value of Sig  $<0.05$  means that there were significant BUN differences between treatment groups.

The results of the statistical analysis continued on the One-way ANOVA test, found that there were differences in the mean BUN levels in the negative control group against the positive control group and each treatment group with a  $p\text{-value}$

$<0.05$ . On the other hand, there were no significant differences in BUN levels in the positive control group for the treatment group and between treatment groups with  $p>0.05$ .

The sample in this study was rat blood serum obtained from research subjects. The analysis was carried out on the serum creatinine level values for each study subject. The average serum creatinine in the negative control group was 0.5189; positive control group 0.5714, and in the treatment group 0.5308. Thus, it could be concluded descriptively that the highest average SC is in the positive control group, namely experimental animals that were induced by urolithiasis without intervention.

The mean value of serum creatinine was then tested for normality using the Shapiro-Wilk analysis because the number of samples was  $<50$ . The mean serum creatinine data between groups were normally distributed ( $p >0.05$ ). Furthermore, Levene's test variability analysis was done. From the results of the homogeneity test output, it was known that all groups had  $p\text{-value}= 0.415$  ( $p\text{-value} >0.05$ ) which means homogeneous data. The analysis was followed by a comparison of the mean serum creatinine levels in each group using the one-way ANOVA parametric test. Based on the ANOVA test results obtained that the SC variable with a  $p\text{-value} >0.05$  means that there was no significant SC difference between treatment groups.

**Table 2.** Comparison of BUN levels in study subjects.

Group	Mean $\pm$ SD	Lower Bound – Upper Bound	P-value Shapiro Wilk	P-value ANOVA
KN (Negative control)	9.89 $\pm$ 2.147	8.24 - 11.54	0.293	<b>0.023*</b>
KP (Positive control)	14.29 $\pm$ 3.147	11.38 - 17.20	0.244	
P (Treatment)	11.22 $\pm$ 3.456	8.57 - 13.88	0.508	

\* significant,  $p\text{ value} <0.05$

**Table 3.** Comparison of BUN levels between treatment groups.

Group	P-value
KN vs KP	<b>0.019*</b>
KN vs P	0.122
KP vs P	0.611

\* significant,  $p\text{ value} < 0.05$

**Table 4.** Comparison of serum creatinine levels in study subjects.

Group	n	Mean $\pm$ SD	P-value Shapiro Wilk	P-value ANOVA
KN (Negative control)	9	0.5189 $\pm$ 4.807	0.143	0.243
KP (Positive control)	9	0.5714 $\pm$ 11.810	0.177	
P (Treatment)	9	0.5111 $\pm$ 4.595	0.476	

There were 3 groups in this study with the negative control group, positive control, and treatment group. After kidney treatment, rats were analyzed histopathologically with hematoxylin-eosin (HE) staining. Observation of the data was carried out by observing degeneration, necrosis, infiltration of interstitial inflammation cells in the renal tubules, interstitial, and glomerulus contained in the cortex or medulla with the magnification of 100x and 400x. The results of microscopic

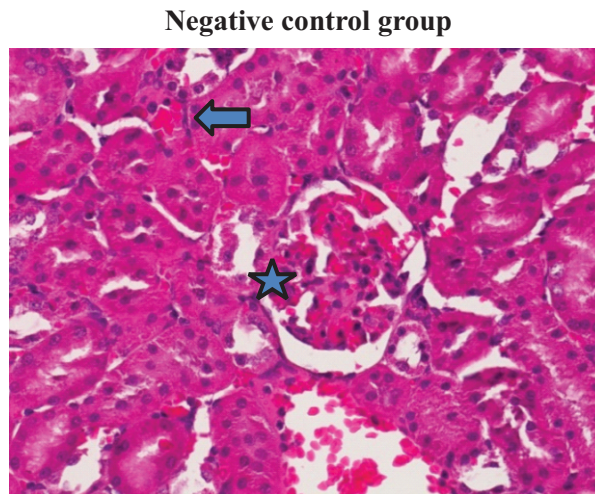
observations were quantified into a combined score used to assess the degree of damage to rat nephrons. The higher the score obtained, the greater the degree of damage to the nephron.

Microscopic images and image capture in this study using a Nikon ECLIPS Ci fluorescence microscope equipped with Nikon Digital SIGHT DS-U3. Data was collected at 100x and 400x magnification.

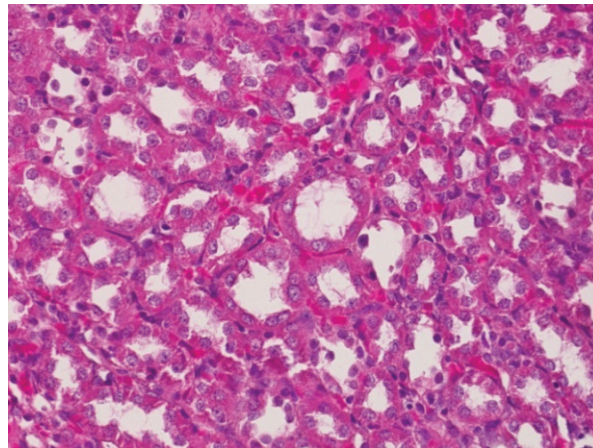
**Table 5.** Comparison of the degree of nephron damage in the study group.

Group	n	Mean $\pm$ SD	Normality	P-value
KN (Negative control)	9	5.83 $\pm$ 1.26	0.474	<b>0.00*</b>
KP (Positive control)	9	10.36 $\pm$ 1.34	0.277	
P (Treatment)	9	5.22 $\pm$ 1.72	0.119	

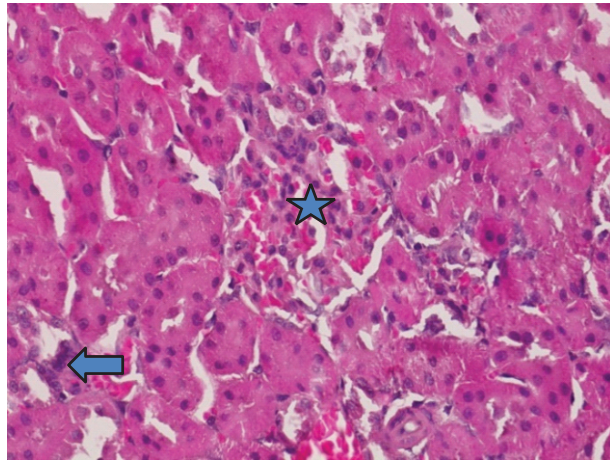
\* significant, p value <0.05



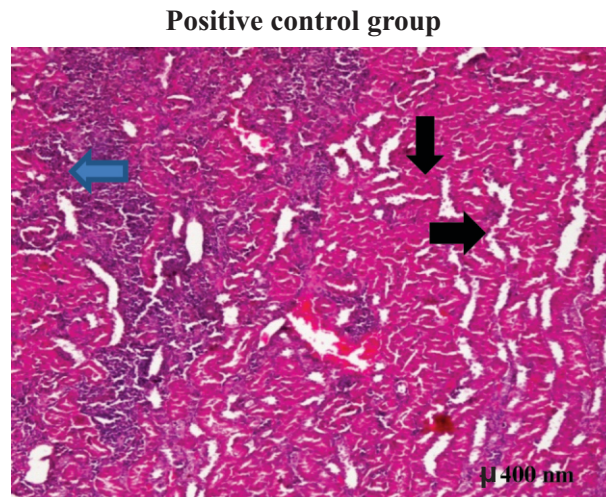
**Figure 1.** Microscopic image of erythrocyte infiltration in interstitial tubules and asterisks showing glomerular enlargement which is not too significant at magnification (400x).



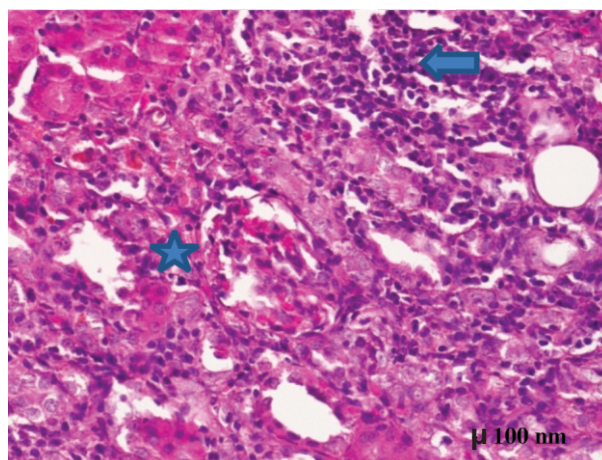
**Figure 2.** Interstitial tubules at magnification (400x).



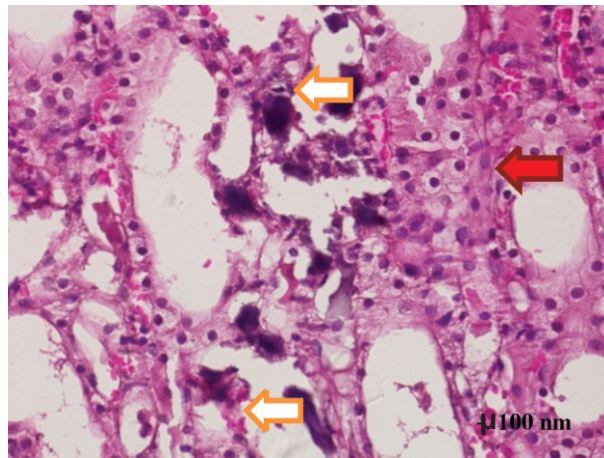
**Figure 3.** Star sign showing glomerular enlargement and blue arrow showing minimal erythrocyte infiltration at magnification (400x).



**Figure 4.** Blue arrows indicate lymphocyte infiltration and black arrows indicate dilation of the tubules with magnification (100x).

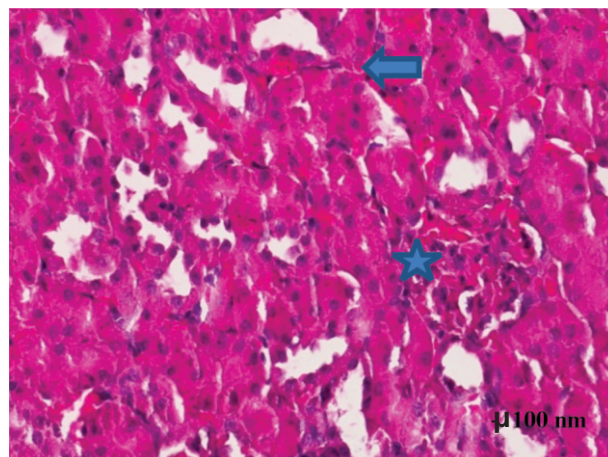


**Figure 5.** Blue arrows show infiltration of lymphocytes and asterisks show enlargement of glomerulus at magnification (400x).

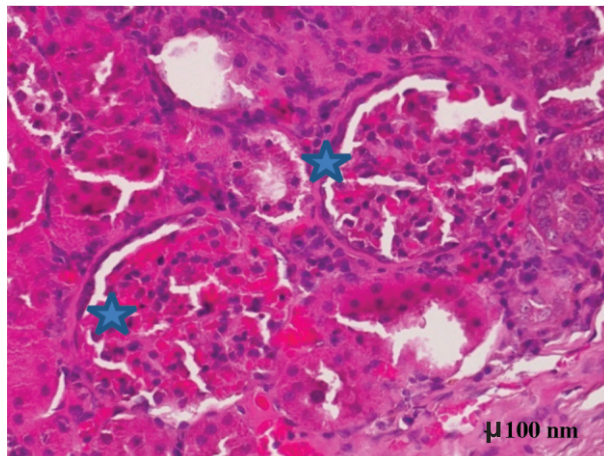


**Figure 6.** White arrows show calcium deposits in the lumen of tubules and red arrows indicate degeneration of tubular cells at magnification (400x).

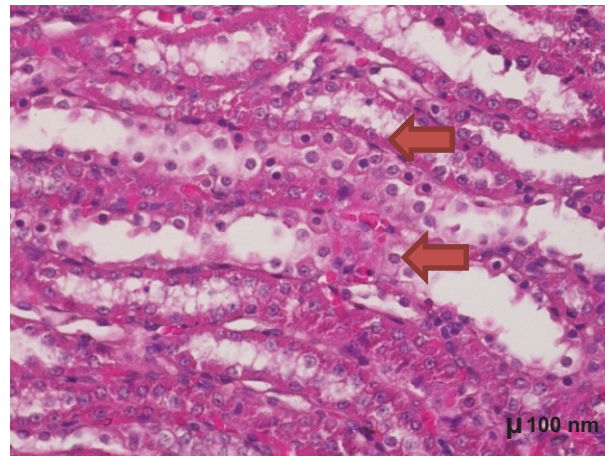
**Treatment group**



**Figure 7.** Blue arrows show erythrocyte infiltration in interstitial tubules and asterisks show glomerular enlargement at magnification (400x).



**Figure 8.** Star sign showing glomerular enlargement at magnification (400x).



**Figure 9.** Red arrows show the degeneration of renal tubular cells at magnification (400x).

**Table 6.** Comparison of degrees of kidney nephron damage between treatment groups.

Comparison of nephron damage between groups	Mean Difference	Confidence interval 95%		P-Value
		Lower bound	Upper bound	
KN Vs KP	4.527*	3.11	5.94	<b>0.001*</b>
KN Vs P	0.611*	-0.81	2.03	0.383
KP Vs P	5.138*	3.71	6.55	<b>0.001*</b>

\* significant, p value <0.05

Normality test was done on the score of the degree of nephron damage obtained in each group. From the Shapiro Wilk normality test, it was found that the degree of damage to renal nephrons was normally distributed and each group was homogeneous with  $p > 0.05$  (Table 5.5). Then a comparative test on the mean (mean) score of the nephron damage is performed using the One Way ANOVA parametric test. The results found a significant difference in the mean between the study groups with a  $p$ -value <0.05 (table 5.5). Variability of data between groups was further analyzed using the Levene test, and there were no significant differences in the variance of data between groups with  $p$ -value >0.05. Therefore, the LSD post hoc test was used to determine differences in nephron damage in each treatment group. The result was a significant difference in nephron damage in the negative control group and positive control with  $p < 0.05$ . Furthermore, a significant difference in kidney damage was found in the positive control group compared to the treatment group with  $p < 0.05$ . However, this study found no statistically significant differences between the negative control group and the treatment group with  $p > 0.05$ .

Treatment with coconut water administration inhibits the deposition of calcium crystals in kidney tissue and reduces and protects against impaired kidney function, as evidenced by a decrease in BUN, and SC levels compared to positive controls. The results showed that coconut water could be a potential candidate for a preventive modality for the occurrence of urolithiasis in white rats of calcium-oxalate-induced Wistar rats.

## DISCUSSION

Renal dysfunction generally increases in the condition of urolithiasis. Increased creatinine and BUN occur when there is dysfunction or kidney damage.<sup>14</sup> This study was carried out to determine whether administration of coconut water would effectively improve kidney dysfunction by examining kidney function parameters/markers such as serum creatinine, blood urea nitrogen (BUN), and histopathological estimation of the degree of kidney damage. This study showed that coconut water administration had the potential to be prevention modality for the occurrence of Urolithiasis in calcium oxalate-induced Wistar rats, by inhibiting

the deposition of calcium crystals in kidney tissue and reducing and protecting from impaired kidney function, as evidenced by a decrease in BUN levels, and SC compared to the positive control.

This study analyzed the effect of the administration of young coconut water (*Cocos nucifera*) on the effects on kidney function related to urinary tract stones in mice. This study used Blood Urea Nitrogen (BUN), and serum creatinine as parameters of kidney function. Descriptive data showed that the average BUN in the positive control group given induction of urolithiasis without intervention was 14.29, while the average BUN in the treatment group was 11.22. By calculation, the average value was lower in the group treated with a coconut water diet, but on the other hand, when analyzed statistically there were no significant differences in BUN levels ( $p > 0.05$ ). Serum creatinine on an average calculation also found lower values in the group treated with coconut water diet (0.5714 in the positive control group, compared to 0.5308 in the treatment group). However, when analyzed statistically there was also no significant difference in serum creatinine levels ( $p > 0.05$ ). This condition showed that there was potential after intervention with coconut water, where BUN values and serum creatinine showed a decrease direction although not statistically significant. Where coconut water was also referred to as having the potential to improve kidney function.<sup>15</sup>

Until now, there has only been one study conducted by Gandhi et al., regarding the benefits of young coconut water in preventing the onset of urolithiasis. In this study, the efficacy of coconut water was evaluated in male Wistar rats with nephrocalcinosis due to ethylene glycol induction. In that study, the dose of coconut water was not determined, and given ad libitum or according to the wishes of the research subjects.<sup>10</sup>

Stone formation in animals that are fed ethylene glycol is caused by a hyperoxaluria condition, which causes an increase in kidney retention and excretion of oxalate.<sup>16</sup> Under hyperoxaluric conditions, oxalate is reported to induce lipid peroxidation and cause kidney damage due to its reaction with polyunsaturated fatty acids in cell membranes. In the study, it was found that lipid peroxidation increased significantly during hyperoxaluria. The increased malondialdehyde (MDA) content might result from an increase in free radicals due to stress conditions in mice undergoing ethylene glycol intoxication.

Administration of coconut water can reduce MDA levels, thereby demonstrating the potential of coconut water as a substance that can reduce free radicals.<sup>10</sup> In addition, young coconut water can also rebalance the activity and expression of antioxidant enzymes (SOD and CAT) which then provide a protective effect against free radicals that induce oxidative stress.<sup>17</sup> The antioxidant effect of young coconut water has also been found to have a protective effect on tissue damage caused by oxalate and crystal deposition.<sup>18</sup> Ethylene glycol administration can increase serum creatinine and urea levels, which cause kidney dysfunction due to urolithiasis. In this condition, young coconut water also plays a role in maintaining kidney function by rebalancing serum urea levels.<sup>10</sup> Administration of young coconut water in animals with urolithiasis prevents the supersaturation of calcium oxalate and reduces deposition in the kidney tubules.<sup>10</sup>

Benefits of young coconut water (*Coco Nucifera*) in preventing the formation of urinary tract stones. In patients with nephrolithiasis due to calcium oxalate, the condition for hypocitraturia is found in 60% of cases.<sup>19</sup> Current medical therapy that has proven effective for hypocitraturia is potassium citrate.<sup>20</sup>

In a study conducted by Patel et al., 2018, it was found that therapy with young coconut water, despite having a relatively low citrate content (2.1 mmol/L), showed a significant increase in urinary citrate excretion from the baseline (average) increased 161 mg/day). This citraturic effect is probably caused by very high total alkali levels (13.8 mEq/L), which is higher than the juice drinks or other non-juice liquids discussed.<sup>21</sup>

There are studies on the impact of water quality on the risk of stone formation. Several studies that are currently available have examined the relationship between water quality and kidney stone formation. Mineral water varies in mineral and electrolyte content between geographical regions, even in the same country. The type of mineral water is determined by the concentration of multivalent cations, especially calcium and magnesium. Hard water is defined as having a higher concentration of calcium carbonate ( $\text{CaCO}_3$ ) than soft water and is found in areas with the geology of limestone and limestone. Hard water is mineral water that contains lots of dissolved minerals (such as calcium and magnesium). Soft water is processed water where the only ion is sodium.<sup>22</sup>

A randomized, double-blind crossover study of 18 types of mineral water, found that

drinking mineral water with a  $\text{Ca}^{2+}$  + 255 mg/L content was associated with a significant increase in urine calcium concentration (although there was no change in oxalate excretion) compared to mineral water - soft water ( $\text{Ca}^{2+}$  + 22 mg/L) or tap water ( $\text{Ca}^{2+}$  + 63.8 mg/L).<sup>23</sup>

Siener et al. showed that mineral water rich in magnesium, calcium, and bicarbonate increased urine pH, as well as concentrations of stone inhibitory compounds such as citrates, which resulted that despite increased urine calcium, calcium oxalate supersaturation was relatively reduced.<sup>24</sup>

Analysis of urine revealed that compared to mineral water, coconut water consumption increased urine citrate by 29%, urine potassium by 130%, and urine chloride by 37%, without significantly affecting urine volume or urine pH. Analysis conducted on coconut water found that although it contained low levels of citrate, it contained high levels of total alkali (13.8 mEq/L). Despite the relatively low citrate content (2.1 mmol/L), coconut water revealed a significant increase in urinary citrate excretion from the start. This is likely due to the very high total alkaline load, which according to their team is mainly a function of the high pH and malate content of coconut water.<sup>15</sup>

Increased citrate levels occur in individuals without a history of previous stones with normal initial citrate values. A similar or greater impact on patients with hypocitraturia with a history of stones has never been tested before. The very high alkaline content of coconut water is also associated with a strong and significant citraturic effect in preventing urinary tract stones.<sup>15</sup>

Providing young coconut water was also found to reduce the amount of crystals in the urine. The results found are also consistent with research conducted by Itoh et al. where green tea could reduce CaOx crystal deposits in rat kidneys with artificial hyperoxaluria conditions due to the administration of ethylene glycol.<sup>11</sup> Administration of young coconut water in animals with urolithiasis prevents the supersaturation of calcium oxalate and reduces deposition in the kidney tubules.<sup>10</sup>

The antioxidant mechanism protects against oxidative injury by oxalate and crystal deposits. Coconut water has the antioxidant potential that can be considered as an additional benefit.<sup>25</sup>

Another study by Balit et al., a preliminary study with a healthy experimental animal model illustrated that giving coconut water intake of 10 mL/kg body weight per day, which was equivalent to the consumption of one coconut per day, was the

optimal dose in terms of maintaining nerve cell density and serum markers from kidney organs.<sup>24</sup>

In this study macroscopic formation of stones could not be observed, but the results of the histopathological analysis in the positive control group contained calcium deposits. This showed that administration of 0.75% ethylene glycol (EG) + 2% ammonium chloride was able to induce stone formation in mice. Different things were found in the treatment group, where administration of 0.75% ethylene glycol (EG) + 2% ammonium chloride and coconut water *ad libitum* resulted in calcium deposits not being observed in the kidney tubules and glomerulus. This indicates that coconut water had a protective function against stone formation. This finding was in accordance with studies by Gandhi et al., Where histopathological analysis showed that crystal deposition induced by EG in kidney cells, and most of the crystal deposition occurs in the kidney tubules, which corroborates the results of other studies report that crystal deposition mainly occurred in tubule.<sup>26</sup> Provision of coconut water in animal models of urolithiasis has also been proven to prevent the supersaturation of calcium oxalate and thereby reduce its deposition in kidney tubules.<sup>10</sup>

Calcium oxalate and calcium phosphate crystals are formed when the concentration of reactant compounds exceeds the limit. Deposits are deposited in the inner interstitium of the medulla in the basement membrane of the Henle loop. Calcium phosphate plaque can enlarge into the surrounding interstitial tissue, or even rupture and lead to the lumen of the tubules, thereby increasing the formation of calcium oxalate stones.<sup>26</sup> Other studies have previously shown that tubular cell injury facilitated the formation and deposition of calcium oxalate crystals (CaOx) in the kidney tubules.<sup>26</sup>

In this study, another parameter observed changed in kidney tissue assessed from the histopathological results. The method of examination of histological changes in this preparation, using the modified Klopffleisch (2013) method. Histopathologically, what was observed was the process of degeneration, necrosis, infiltration of interstitial inflammation cells in the renal tubules, interstitial, and glomerulus found in the cortex or medulla. The degree of kidney damage assessed in this study was observed based on the inflammatory response that occurs in the kidney tubules and glomerulus. Changes in renal histopathological features in this study also became a reference to the effect of coconut water on rat

nephrons. Through histopathological features, there was the enlargement of the glomerulus and degeneration of tubular cells. From the results of microscopy observations, as for tubular damage around  $\mu 100$  nm. Reference to the formation of free radicals in this study was inflammation caused by changes in cellular free radicals. This might explain the picture of inflammatory microscopy in the administration of young coconut water to reduce chronic inflammation in the tubules and kidneys. This could show the benefits of coconut water in protecting kidney function from damage caused by stones.

In the end, until now there have been no clinical studies that show coconut water to prevent or treat kidney stones. It's just that research has shown possible benefits that are limited to rat models and studies in people that show that intake of large amounts of coconut water increases urinary citrate levels, a compound that can help inhibit kidney stone formation.

## CONCLUSION

Based on the results of the study, it was concluded that coconut water could be a potential candidate for a preventative modality for the occurrence of urolithiasis in calcium oxalate induced-white rat Wistar strains, which:

1. There were significant differences in the mean lower BUN levels in mice receiving 0.75% ethylene glycol (EG) + 2% ammonium chloride induction and coconut water administration ad libitum, compared with the positive control group.
2. There was a lower mean difference in SK levels in rats receiving 0.75% ethylene glycol (EG) + Ammonium chloride 2% induction and intervention with coconut water administration ad libitum, compared with the positive control group. But this difference was not statistically significant.
3. Statistically, there was a significant difference in kidney damage through histopathological observation (dilation and degeneration of tubules and glomerulus) in mice that get mineral water + 0.75% ethylene glycol + 2% ammonium chloride.
4. Descriptively, there was calcium deposits in the kidney tubules in the control group which received mineral water + ethylene glycol 0.75% + Ammonium chloride 2%, the same matter was

not found in the control group and the treatment group.

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