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# Ethanol Extract of Rosa Damascena Effects the Kidney Crystal Deposit in Wistar Rats

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**ABSTRACT**

**This research aims to show that the elixir of the ethanol extract of Rosa damascena on the solubility of urine calcium oxalate crystal in Wistar rats. The experimental rats were divided into six groups: the control group (K0); ethylene glycol 0.75% and ammonium chloride 2% as an inducer (K-); inducer and Batugin elixir (K+); inducer and the elixir of the ethanol extract of Rosa damascena 250 mg/kg body weight (P1); 500 mg/kg body weight (P2) and 1000 mg/kg body weight (P3). The treatment was carried out for 28 days by examining urine crystals; urine pH was carried out on days 14 and 28, rat body weight measurements were carried out on days 14 and 28. At the end of the treatment period, the rats were necropsied using a combination of ketamine xylazine, and their kidneys were taken. Results showed that the elixir of ethanol extract of Rosa damascena was able to dissolve calcium oxalate crystals with the best dose of 500 mg/kg BW based on the weight gain, decreased kidney weight ratio, decreased concentration of creatinine and urea, and decreased number of kidney crystal deposits.**

**Keywords:** Elixir; R. Damascena; Calcium oxalate; Kidney crystal deposit; Ethanol extract.

## INTRODUCTION

Modern treatments for kidney stones have been widely used, such as SWL (Shock Wave Lithotripsy (SWL), Percutaneous Neprhrolithotripsy (PNL), and Retrograde Intra Renal Surgery (RIRS) [1]. However, modern medicine certainly requires relatively expensive costs, so research is needed from natural ingredients for traditional medicine as a more affordable alternative. Several previous studies with traditional plants have researched the treatment of kidney stones through the nephroprotective effect of ethanolic extract of balakka [2] the urolithiasis effect of ethanolic extract of Portulaca oleracea [3]. From these studies, it was found that these plants have effectiveness in treating kidney stones.

Rosa damascena is a popular ornamental plant commodity and is widely cultivated in Indonesia [4]. Rosa damascena promotes blood circulation, normalizes the menstrual cycle, antiinflammatory, eliminates swelling, and neutralizes toxins. Some of the chemical compounds in Rosa damascena include citral, citronellol, geraniol, linalool, nerol, eugenol, phenyl ethyl alcohol, farnesol, anisaldehyde [5]. Rosa damascena also contains chemical compounds terpenoids, glycosides, flavonoids, and anthocyanins [6]. Based on this explanation, the authors are interested in examining the effect of the elixir of the ethanol extract of Rosa damascena on the solubility of urine calcium oxalate crystal in Wistar Rats.

## MATERIALS AND METHODS

Materials were used in this study are pH indicator, oral probe, rat cage, rat scale, analytical balance, light microscope, dropper pipette, test tube, funnel, filter paper, blender, measuring cup, beaker glass, a set of surgical instruments, rotary evaporator, syringe, microhematocrit, Eppendorf tube, micropipette, centrifuge, microplate reader, spectrophotometer.

Phytochemical screening on Rosa damascena extract included examining the secondary chemical metabolites of alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and steroids [6], [7].

The division of groups and treatment in this study were:
As many as 24 rats were randomly divided into six groups so that in one group, there were four rats. The group divisions are:

a. Group K0 = consisted of 4 male Wistar rats who were given drinking plain water.
b. Group K- = 4 male Wistar rats induced with ethylene glycol 0.75% and ammonium chloride 2%
c. Group K+ = 4 male Wistar rats induced with ethylene glycol 0.75% and ammonium chloride 2% and given Batugin elixir orally.
d. Group P1 = 4 male Wistar rats induced with ethylene glycol 0.75% and ammonium chloride 2% and given the elixir of the ethanol extract of Rosa damascena orally at a dose of 250 mg/kg body weight.
e. Group P2 = 4 male Wistar rats induced with ethylene glycol 0.75% and ammonium chloride 2% and given the elixir of the ethanol extract of Rosa damascena orally at a dose of 500 mg/kg body weight.
f. Group P3 = 4 male Wistar rats induced with ethylene glycol 0.75% and ammonium chloride 2% and given the elixir of the ethanol extract of Rosa damascena orally at a dose of 1000 mg/kg body weight.

The treatment was carried out for 28 days by examining urine crystals; urine pH was carried out on days 14 and 28, rat body weight measurements were carried out on days 14 and 28. At the end of the treatment period, the rats were necropsied using a combination of ketamine xylazine, and their kidneys were taken. Observations were made on the ratio of kidney weight to body weight of rats and measurements of blood serum creatinine and urea levels using a spectrophotometer.

The data obtained are quantitative and qualitative. Quantitative data in the form of urine pH, the severity of calcium oxalate dihydrate (COD) crystals, body weight, the ratio of kidney weight to the importance of rats, blood serum creatinine, and urea levels. Qualitative data in the form

of the chemical content of Rosa damascena extract and kidney histopathology. Statistical tests were carried out with SPSS on quantitative data; if the data were homogeneous, the Anova test was carried out. The Kruskal Wallis test is carried out [8].

## RESULTS AND DISCUSSION

### Phytochemical content and pH analysis

By preliminary screening of phytochemical tests, Rosa damascena extract consists of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids.

The pH examination of urine was carried out on days 14 and 28, as shown in Table 1:

**Table 1. The urine pH examination on day 14 and 28**

| Groups | Day-14 | Day-28 |
|---|---|---|
| K0 | 6.3 ± 0.52 | 7.6 ± 0.89 |
| K- | 5.5 ± 0.58 | 7.8 ± 0.45 |
| K+ | 6.5 ± 1.00 | 7.0 ± 1.41 |
| P1 | 5.5 ± 0.58 | 6.5 ± 1.00 |
| P2 | 6.0 ± 0.00 | 6.8 ± 1.64 |
| P3 | 6.0 ± 0.00 | 5.8 ± 0.96 |

The results of examining the pH of the rat urine on day 14 in all groups showed that the pH was in the range of 5.5-6.5. This indicates that calcium oxalate has begun to form. Crystal formation can be influenced by pH [9]. Calcium oxalate crystals are formed at a pH of 5.0-6.5 [3], which is the condition of urine pH below 7 [10]. On day 28, the K+, P1, P2 groups showed urine pH values close to normal urine pH except for the P3 group. The group that was not given the elixir of the ethanol extract of Rosa damascena(K- group) and the control group (K0) showed a slightly alkaline pH change, but still within the average urine pH value of rats. The pH value of normal urine of rats ranges from 7.3 to 8. The pH value of urine is determined by acid and alkaline regulation in the kidneys [11].

### Rat body weight analysis

The rat's body weight was checked on days 0, 14, and 28, with the results in Table 2:

**Table 2. Bodyweight of rats on days 0, 14th and 28th**

| Groups | Day-0 | Day-14th | Day-28th |
|---|---|---|---|
| K0 | 247.50 ± 29.76 | 255.83 ± 26.38 | 247.17 ± 29.16 |
| K- | 264.00 ± 18.38 | 232.40 ± 22.36 | 238.20 ± 15.77 |
| K+ | 233.00 ± 36.91 | 235.00 ± 54.99 | 248.80 ± 67.14 |
| P1 | 239.50 ± 28.70 | 216.75 ± 28.69 | 225.75 ± 36.59 |
| P2 | 269.83 ± 21.53 | 236.33 ± 24.40 | 252.40 ± 24.71 |
| P3 | 271.67 ± 28.72 | 238.20 ± 40.30 | 258.00 ± 37.04 |


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Weighing was carried out to observe the general clinical condition of each experimental animal. Day 0 is the day after adaptation for a week before the mice received treatment. On day 14, the K0 and K+ groups experienced weight gain, while the K-, P1, P2, and P3 groups experienced weight loss. All treatment groups (groups K-, K+, P1, P2, P3) experienced weight gain at the end of the treatment period (day 28) except the control group (K0). Bodyweight that is relatively constant or has a gentle increase can describe the condition of a healthy and stable animal. Conversely, a decrease or change in body weight can tell the state of the experimental animal that is experiencing health problems. Weight gain is thought to be due to the restoration of body condition related to a decrease in the concentration of crystal components in the urine, antioxidant and anti-free radical activity [12].

## Urine crystal analysis

Urine crystals were examined using a light microscope at magnifications of 100 and 400 times. The types of crystals found in all animal groups can be seen in Table 3 and Figure 1:

**Table 3. Type of Urine Crystal on day 14th and 28th**

| Groups | Day-14th | Day-28th |
|---|---|---|
| K0 | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| | $Ca_3(PO_4)_2$ crystals | $Ca_3(PO_4)_2$ crystals |
| K- | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| | Phosphate amorphous crystals | Phosphate amorphous crystals |
| K+ | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| | Phosphate amorphous crystals | Phosphate amorphous crystals |
| | $Ca_3(PO_4)_2$ crystals | $Ca_3(PO_4)_2$ crystals |
| | $2Ca_3(PO_4)_2$ crystals | $2Ca_3(PO_4)_2$ crystals |
| P1 | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| | Phosphate amorphous crystals | Phosphate amorphous crystals |
| | $Ca_3(PO_4)_2$ crystals | $Ca_3(PO_4)_2$ crystals |
| | $2Ca_3(PO_4)_2$ crystals | $2Ca_3(PO_4)_2$ crystals |
| P2 | Phosphate amorphous crystals | Phosphate amorphous crystals |
| | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| P3 | $CaC_2O_4.H_2O$ crystals | $CaC_2O_4.H_2O$ crystals |
| | $CaC_2O_4.2H_2O$ crystals | $CaC_2O_4.2H_2O$ crystals |
| | $Ca_3(PO_4)_2$ crystals | $Ca_3(PO_4)_2$ crystals |

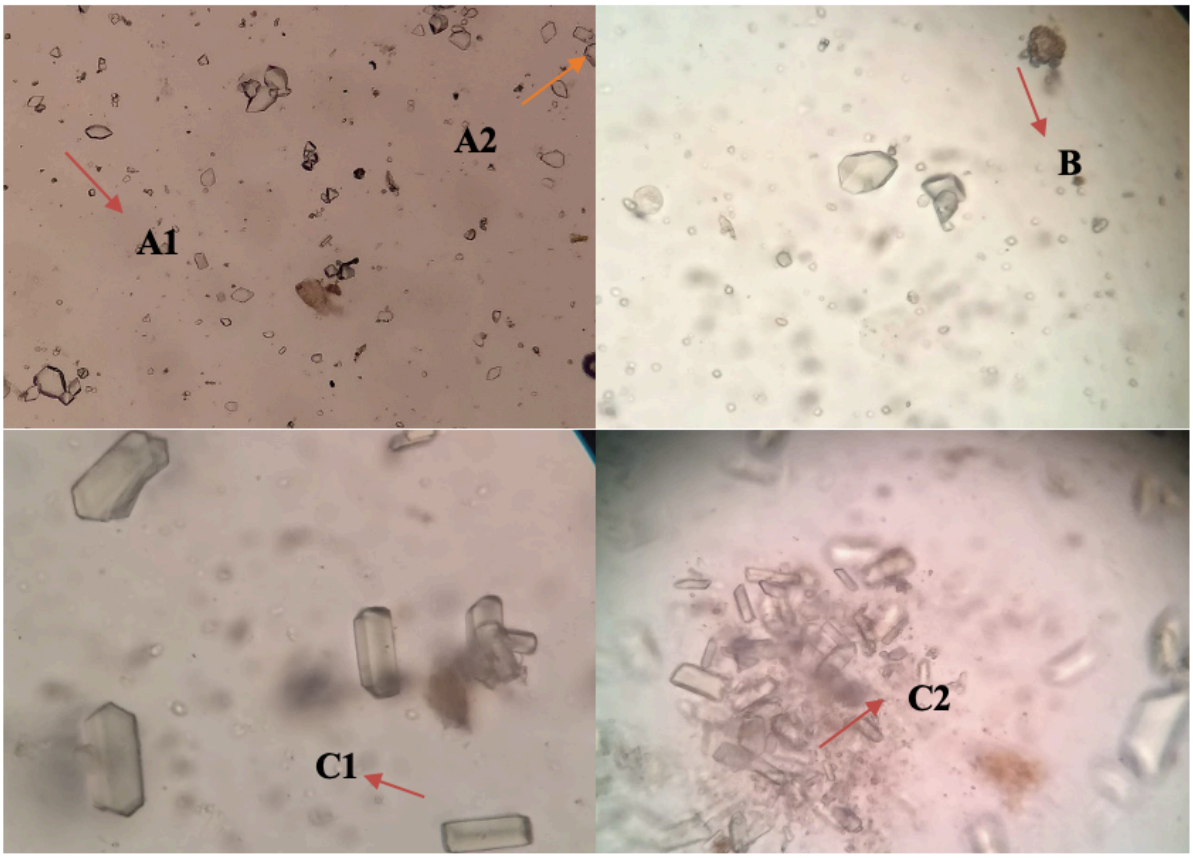


**Figure 1. Microscopic observation of urine crystals formation**

From the Figure 1, the A1 is calcium oxalate monohydrate crystals; A2 is calcium oxalate dihydrate crystals; B is Phosphate amorphous crystals; C1 is calcium phosphate crystals; C2 is dicalcium phosphate crystals.

### Quantity of urinary calcium oxalate dihydrate (COD) crystals

The quantity of urine crystals have been counted to measure the number of crystals, this may relate to the solubity index and severity level of each groups after treatment as shown in Table 4:

**Table 4. Number of urine crystals on day 14th and 28th**

| Groups | Day-14 | | Day-28 | |
|---|---|---|---|---|
| | Number of crystals | Scoring | Number of crystals | Scoring |
| K0 | 1.33 ± 0.52 | + | 1.40 ± 0.55 | + |
| K- | 4.75 ± 0.50 | + | 8.00 ± 1.00 | ++ |
| K+ | 1.75 ± 0.50 | + | 3.20 ± 0.45 | + |
| P1 | 1.50 ± 0.58 | + | 4.00 ± 0.82 | + |
| P2 | 1.50 ± 0.71 | + | 1.80 ± 0.45 | + |
| P3 | 2.25 ± 0.96 | + | 3.00 ± 1.15 | + |

In all treatment groups (groups K0, K-, K+, P1, P2, and P3), almost the same types of crystals were found. Crystalline calcium oxalate monohydrate (calcium oxalate monohydrate or COM)


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or whewellite, calcium oxalate dihydrate (calcium oxalate dihydrate or COD), or weddellite, calcium phosphate (calcium phosphate or CaP), or amorphous phosphate are the crystals found. The type of crystal found is inorganic urine sediment and is classified as a standard crystal type influenced by the level of acidity or pH [13]. Calcium oxalate stones are mostly monohydrate or dihydrate as the main component of kidney calcium stones and are the most common in urolithiasis. Electrostatic interactions between calcium ions and oxalate ions at high concentrations in the renal tubular fluid form calcium crystals [14]. Phosphate amorphous crystals are often found together with calcium phosphate crystals. Amorphous phosphate crystals are shaped like small, colorless granules and are distinguished from amorphous urate based on pH [15].

Administration of 0.75% ethylene glycol and 2% ammonium chloride can induce hyperoxaluria and calcium oxalate deposition in the kidneys. Oxalate induces lipid peroxidation and causes damage to kidney tissue which reacts with unsaturated fatty acids in cell membranes [16]. A more significant risk factor than hypercalciuria in the pathogenesis of kidney stones is hyperoxaluria because urinary oxalate levels have a more substantial effect than calcium. However, the contributing factor for the nucleation of calcium oxalate or calcium phosphate deposition and subsequent crystal growth is an increase in urinary calcium [17].

Calcium oxalate crystals are usually excreted freely, but in pathological conditions, calcium oxalate crystals can develop into stones if they are kept in the kidneys. The severity of the crystals can be seen from the observed data on the number of calcium oxalate dihydrate (COD) crystals. On examination of the severity of the crystals, namely calcium oxalate dihydrate crystals, it was seen that all groups (K0, K-, K+, P1, P2, P3) showed the same number of crystals ranging from 1-5 crystals (+) both on the 14th and 14th-day observations. 28. However, on the 28th day of observance, the number of calcium oxalate dihydrate crystals found in the K- group increased from the 14th day of observation in the range of 6-20 crystals (++). The number of calcium oxalate crystals in the K+, P1, and P3 groups was less than the K- group on the 14th and 28th-day observations. On the 28th day of observance, the amount of calcium oxalate dihydrate in the P2 group was less than the K+, P1, and P3 groups. This indicated that the P2 group could dissolve crystals and that there was an inhibitory activity of calcium oxalate crystal formation for 28 days based on the number of crystals found. The P1 and P3 groups can also dissolve calcium oxalate crystals, although the more soluble crystals in the P2 group.

**Kidney weight ratio**

The weight ratio (w/w) of kidney as shown in table 5:

**Table 5. Kidney Weight Ratio of Rats**

| Groups | Kidney weight | Kidney weight ratio (g/100g) |
|---|---|---|
| K0 | 0.84 ± 0.11 | 0.34 ± 0.03 |
| K- | 1.08 ± 0.17 | 0.46 ± 0.09 |
| K+ | 1.01 ± 0.26 | 0.40 ± 0.04 |
| P1 | 1.12 ± 0.45 | 0.55 ± 0.32 |
| P2 | 1.19 ± 0.16 | 0.47 ± 0.07 |
| P3 | 1.09 ± 0.33 | 0.44 ± 0.07 |

The K+ and P3 groups had a smaller kidney weight ratio than the K- group compared to the P1, P2 groups. Kidney stones can cause an increase in kidney weight; this is due to high mineral levels in the kidneys and swelling due to an inflammatory reaction. Rats will experience decreased bodyweight due to high doses of ethylene glycol poisoning and increased kidney weight, and the ratio of kidney weight to body weight [18]. The activity of flavonoids as an anti-inflammatory can reduce kidney weight [19].

### Creatinine and urea levels

The results of measuring blood serum creatinine and urea levels can be seen in Table 6.

**Table 6. Blood serum creatinine and urea levels**

| Groups | Creatinine level (mg/dL) | Ureum level (mg/dL) |
|---|---|---|
| K0 | 0.54 ± 0.10 | 28.00 ± 11.80 |
| K- | 0.61 ± 0.11 | 28.58 ± 9.40 |
| K+ | 0.63 ± 0.13 | 22.12 ± 14.00 |
| P1 | 0.63 ± 0.15 | 26.23 ± 9.92 |
| P2 | 0.57 ± 0.07 | 20.33 ± 2.60 |
| P3 | 0.63 ± 0.03 | 22.95 ± 15.95 |

Creatinine analysis, is one of the screening tests for kidney function. The condition of nephrons in the urinary system can be observed through changes in serum creatinine concentrations. Creatinine is a product of creatine metabolites whose levels are constant in blood and urine. This will change if there is an injury to the tubule due to kidney stones [20]. The P2 group showed lower creatinine levels than the control group (K0) and the K- group compared to the K+, P1, and P3 groups. Creatinine levels in all groups were within the normal range of creatinine levels. Normal blood creatinine levels for normal male white rats were 0.20-0.89 mg/dL [21].

Urea and other nitrogen-rich waste products will normally be excreted from the blood vessels through the kidneys, so an increase in urea levels can indicate a failure of kidney function [22]. The K+, P1, P2, and P3 groups showed lower urea levels than the control group (K0) and the K- group. Urea levels in all groups were within the normal range of urea levels. Normal blood urea levels for normal male white rats were 15.00-21.00 mg/dL [21]. Giving the elixir of the ethanol extract of Rosa damascena, which contains flavonoids, is thought to increase urination and electrolyte expenditure in normotensive rats. Glomerular filtration rate (GFR) showed a significant increase after flavonoid administration. Small changes in glomerular filtration or tubular reabsorption can increase relatively large changes in urinary excretion because high GFR can make the kidneys remove waste products from the body quickly [23].

## CONCLUSION

This research showed the potential of the elixir of the ethanol extract of R. Damascena extract on dissolving calcium oxalate crystals induced by ethylene glycol 0.75% and ammonium chloride 2%. The best dosage is 500 mg/kg BW 500 mg/kg BW based on the analysis of weight gain, decreased kidney weight ratio, decreased concentration of creatinine and urea, and decreased the number of kidney crystal deposits. This research suggested the potential impact of R. Damascena extract for kidney and urinary tract recovery and further researchs on pharmacodynamics and pharmacokinetics are encouraged.


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