

The Effect of Elixir of the Ethanol Extract of *Rosa damascena* on the Solubility of Urine Struvite Crystal in Wistar Rats

Kistia Maryanti

Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Chrismis Novalinda Ginting

Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Linda Chiuman

Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Sahna Ferdinand Ginting

Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

ABSTRACT

Kidney stones are hard and solid masses formed by crystals in the urinary tract. Hence it should be treated appropriately. The use of *Rosa damascena* might be an option to help the recovery of the kidney and urinary tract. This study aims to show the potential of the elixir of the ethanol extract of *Rosa damascena* on the solubility of urine struvite crystal in Wistar rats. The experimental rats were divided into six groups: the control group (K0); magnesium oxide 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. Observations were made on the ratio of kidney of rats (w/w) and measurements of blood serum creatinine and urea levels using a spectrophotometer. The elixir of *Rosa Damascena*'s ethanolic extract demonstrated the potential of extract administration by raising weight gain, diminishing kidney weight ratio, reducing creatinine and blood urea concentrations, and lowering the number of kidney crystal deposits. The elixir of the ethanol extract of *Rosa damascena* has shown its potential to dissolve struvite crystals with the best dose of 250 mg/kg BW based on the number of urine crystals.

Keywords: Struvite crystal; *R. Damascena*; kidney crystal deposit; ethanol extract.

INTRODUCTION

Struvite stones are also known as infection stones or magnesium, ammonium, phosphate stones. These stones are associated with urea-splitting bacterial infections (*Proteus mirabilis*, *Klebsiella pneumonia*, *Pseudomonas*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) [1]. Urea-breaking bacteria can make urine alkaline by hydrolyzing urea into ammonia, thus

facilitating the formation of magnesium ammonium phosphate (MAP) stones and carbonate apatite in an alkaline environment [2].

Modern management of kidney stones can be done using Extracorporeal Shockwave Lithotripsy (ESWL), Percutaneous Nephro Litholapaxy (PCNL), open surgery, and conservative therapy or expulsive medical therapy (TEM) [3]. However, modern medicine requires relatively more expensive costs, so research is needed from natural ingredients for traditional medicine at a more affordable price. Research on the treatment of kidney stones has been carried out by several previous researchers with conventional plants that have a nephroprotective effect on the ethanolic extract of balakka fruit [4] anticalculi activity of cucumber juice [5]. From the studies that have been done, it was found that these plants have effectiveness in treating kidney stones.

Roses are ornamental plants that have many benefits and uses in the form of extracts and oils [6]. Roses contain geraniol and citronellol. Citral, linalool, nerol, eugenol, phenyl ethyl alcohol, farnesol, nonialdehyde [7]. Rose flowers contain terpenoids, glycosides, flavonoids, and anthocyanins [8]. The essential oils in roses are damascenone, damascone, and ionone, which are obtained from the degradation of carotenoids. Roses also contain phosphorus, potassium, calcium, magnesium, sodium, iron, copper, manganese, zinc, and boron [9]. Some of the pharmacological activities of the rose plant are as an antioxidant and hepatoprotective [10], analgesic, hypnotic, anti-spasmodic, anti-inflammatory [8], antidiabetic [11], anti-microbial, anti-cancer [12]. Some reports on the nephroprotective effects of *Rosa Damascena*, such as rose petals [13] and mill flowers [14], have been reported. To the best of our knowledge, the potential use of elixir of ethanolic extract of *Rosa damascena* to fight against kidney stones is still rarely found.

In this study, we will evaluate the elixir effect of the ethanolic extract of *Rosa damascena* on urinary struvite crystal solubility and kidney histopathology in Wistar rats induced with magnesium oxide. This study might be helpful in the development of new herbal medicine to cure kidney stones recently.

MATERIALS AND METHODS

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 were among the materials used in this study. The secondary chemical metabolites, including alkaloids, flavonoids, glycosides, tannins, saponins, terpenoids, and steroids, were examined by phytochemical screening on *Rosa damascena* extract [15], [16].

Experimental rats were randomly divided into six groups; each group had four rats. The group divisions are:

- a. Group K0 = consisting of 4 male Wistar rats who were given drinking plain water.
- b. Group K- = 4 male Wistar rats induced with magnesium oxide 0.4%
- c. Group K+ = 4 male Wistar rats induced magnesium oxide 0.4% and given Batugin elixir orally.
- d. Group P1 = 4 male Wistar rats induced with magnesium oxide 0.4% 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 magnesium oxide 0.4% 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 magnesium oxide 0.4% 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.

Quantitative data in the form of urine pH, the number of struvite crystals, body weight, the ratio of the kidney (w/w) 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 also carried out [17].

RESULTS AND DISCUSSION

Phytochemical content and pH analysis

The content in *Rosa damascena* extract consists of alkaloids, flavonoids, glycosides, saponins, tannins, and triterpenes/steroids. The pH analysis on days 14 and 28 are shown in Table 1.

Table 1. The urine pH examination on days 14 and 28

Group s	Day-14	Day-28
K0	7.2 ± 0.75	8.8 ± 0.41
K-	6.5 ± 0.55	8.2 ± 0.75
K+	6.8 ± 0.75	9.2 ± 0.41
P1	6.4 ± 0.55	7.8 ± 1.10
P2	6.3 ± 0.82	8.2 ± 1.17
P3	6.7 ± 0.82	9.0 ± 0.89

The results of the examination of the pH of the urine of rats on day 14 in all groups showed a pH < 7 except for the K0 group, which was 7.2 + 0.75. On day 28, all groups showed an alkaline pH outside the normal urine pH value of rats, namely 7.3-8, except for the P1 group with a pH value of 7.8 + 1.10. A pH value > 7 facilitates the formation of struvite crystals [18]. Acid-base regulation in the kidney determines the pH value [19].

Rat body weight analysis

The rat's body weight was measured on days 0, 14, and 28, as shown in Table 2.

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

Groups	Day-0	Day-14th	Day-28th
K0	241.83 $\pm$ 32.69	241.50 $\pm$ 20.89	237.50 $\pm$ 24.59
K-	229.67 $\pm$ 31.74	240.00 $\pm$ 30.49	222.83 $\pm$ 25.14
K+	240.17 $\pm$ 23.84	255.83 $\pm$ 37.62	238.33 $\pm$ 16.32
P1	243.33 $\pm$ 27.66	229.67 $\pm$ 22.55	237.33 $\pm$ 31.58
P2	220.67 $\pm$ 24.13	220.67 $\pm$ 21.00	215.20 $\pm$ 19.01
P3	246.17 $\pm$ 16.87	251.50 $\pm$ 41.05	250.00 $\pm$ 30.88

Weighing was conducted to see how each experimental animal was doing overall health. Day 0 refers to the day after the mice had adapted for a week before receiving treatment. Weight increase was observed in the K-, K+, and P3 groups on day 14, but weight loss was observed in the P1 group. Except for group P1, which gained weight after the treatment period (day 28), all treatment groups (K0, K-, K+, P2, P3) lost weight at the end of the treatment period (day 28). The status of a healthy and stable animal can be described by its body weight being generally constant or gradually increasing. A drop or change in body weight, on the other hand, might represent the status of an experimental animal suffering from health difficulties. The restoration of physical condition is assumed to be attributed to a decrease in the concentration of crystal components in the urine and antioxidant and anti-free radical action [20].

Urine crystal analysis

A light microscope was used to observe urine crystals at magnifications of 100 and 400 times. Table 3 and Figure 1 show each animal group's different kinds of crystals.

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

Groups	Day-14th	Day-28th
K0	Struvite crystals	Struvite crystals
K-	Struvite crystals	Struvite crystals
K+	Struvite crystals	Struvite crystals
P1	Struvite crystals	Struvite crystals
P2	Struvite crystals	Struvite crystals
P3	Struvite crystals	Struvite crystals

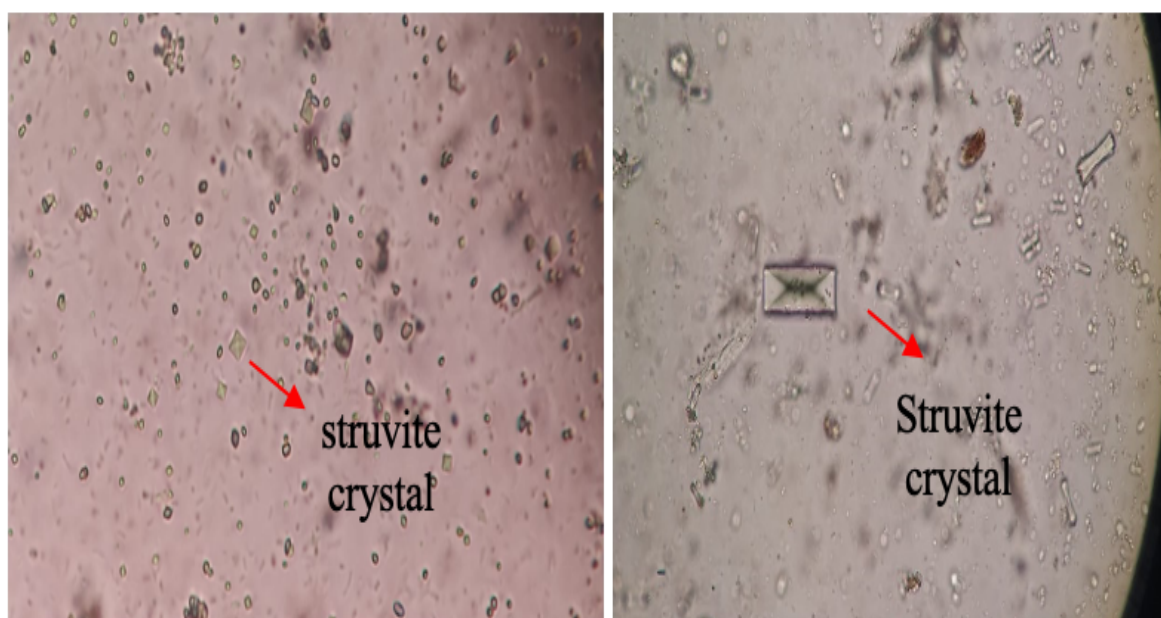


Figure 1. The formation of urine crystals under a microscope

Number of urinary struvite crystals

The amount of the struvite crystal is shown in Table 4.

Table 4. Examination number of urinary struvite crystals

Groups	Day-14	of	Day-28	of
	Number crystals		Number crystals	
K0	7.50 + 2.35	++	6.83 + 2.71	++
K-	31.50 + 14.47	+++	31.50 + 16.08	+++
K+	14.17 + 5.49	++	7.33 + 4.23	++
P1	17.67 + 3.50	++	7.17 + 2.71	++
P2	19.40 + 8.08	++	8.60 + 2.88	++
P3	22.50 + 10.96	++	12.33 + 9.20	++

The same type of crystal was found in all treatment groups, namely struvite crystals. Struvite stones are formed by supersaturation of urine, where they grow into larger particles and turn into crystals. If deposited in the urinary tract, larger crystals can form into struvite stones. In humans, the enzyme urease converts urea into ammonia, and carbon dioxide is reduced to carbonate, increasing urine pH and forming crystals [21], [22]. Struvite is usually prepared by crystallizing three components: magnesium, ammonium, and phosphate [23].

On examination of the severity of crystals, namely struvite crystals, it was seen that all groups showed the same range of crystal numbers, namely 6-20 crystals (++) on both the 14th and 28th days of observation. However, on the 28th day of observation, the number of struvite crystals found in the K-group increased from the 14th-day observation, namely > 20 crystals (+++). The number of struvite crystals in the K+, P1, P2, and P3 groups was less than the K-group on the 14th and 28th-day observations. On the 28th day of observation, the amount of

calcium oxalate dihydrate in the P1 group was less than the K+, P1, and P3 groups. This indicated that the P1 group could dissolve crystals and that there was an inhibitory activity of struvite crystal formation for 28 days based on the number of crystals found. The P2 and P3 groups can also dissolve struvite crystals, although the more soluble crystals in the P1 group. The ethanolic extract of roses contains flavonoid compounds; flavonoids have a role in dissolving struvite crystals and have a urolithiasis effect [24].

Kidney to rat weight ratio

The ratio of kidney weight to rat weight can be seen in Table 5.

Table 5. Kidney to Weight Ratio of Rats

Groups	Kidney weight	Kidney weight ratio (g/100g)
K0	0.75 + 0,10	0.32 + 0,04
K-	0.73 + 0.09	0.33 + 0,02
K+	0.82 + 0,08	0.34 + 0,02
P1	0.81 + 0,12	0.34 + 0,02
P2	0.70 + 0,10	0.33 + 0,05
P3	0.85 + 0,10	0.34 + 0,02

All groups had a kidney weight ratio value more significant than the control group (K0). 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 an increase in kidney weight and the ratio of kidney weight to body weight due to the induction of magnesium oxide, which forms struvite crystals [25].

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 level measurement results

Groups	Creatinine level (mg/dL)	Ureum level (mg/dL)
K0	0.19 ± 0.05	14.38 ± 5.90
K-	0.21 ± 0.04	13.85 ± 2.99
K+	0.18 ± 0.03	11.20 ± 2.15
P1	0.21 ± 0.04	12.74 ± 3.14
P2	0.44 ± 0.08	14.45 ± 7.00
P3	0.40 ± 0.07	11.43 ± 1.77

One of the screening tests for the renal function is creatinine analysis. Changes in serum creatinine concentrations can be used to assess the health of nephrons in the urinary system. Creatinine is a metabolite of creatine that has stable amounts in the blood and urine. This will alter if the tubule is injured due to kidney stones [26]. Compared to the K+, P1, and P3 groups, the P2 group had lower creatinine levels than the control group (K0) and the K- group. All of

the groups had creatinine levels that were within the normal range. Normal male white rats had blood creatinine values of 0.20-0.89 mg/dL [27].

Because the kidneys generally eliminate urea and other nitrogen-rich waste products from the blood vessels, a rise in urea levels can suggest renal failure [28]. Urea levels were lower in the K+, P1, P2, and P3 groups than control (K0) and K- groups. All of the groups' urea levels were within the usual range. Normal male white rats had blood urea values of 15.00-21.00 mg/dL [29]. In normotensive rats, an elixir made from an ethanol extract of *Rosa damascena*, which includes flavonoids, stimulates urine and electrolyte expenditure. Following flavonoid delivery, the glomerular filtration rate (GFR) increased significantly. Because a high GFR causes the kidneys to eliminate waste materials from the body quickly, small changes in glomerular filtration or tubular reabsorption can result in quite substantial changes in urine excretion [30].

CONCLUSION

The elixir of *Rosa damascena* ethanol extract has the effect of dissolving struvite crystals in wistar rats induced by 0.4 percent magnesium oxide. This study revealed that *R. Damascena* extract might help the kidney and urinary tract healing. Further studies on the pharmacokinetics and safety assessment of the new developed herbal drugs using *Rosa Damascena*.

References

- Rasyid N, Kusuma DGW & Atmoko W. Panduan penatalaksanaan klinis batu saluran kemih. Edisi Pertama. Ikatan Ahli Urologi Indonesia (IAUI). 2018.
- Halim P, Girsang E, Nasution AN, Lister INE & Lie S. Nephroprotective effect of ethanolic extract of balakka (*Phyllanthus Emblica* L.) on rats induced ethylene glycol and ammonium chloride. *Indonesian Journal of Pharmaceutical and Clinical Research (IDJPCR)*. 2019; 2(2): 36-42.
- D.V. Kishore, Fouzia Moosavi, DR. R. K. Varma. Effect of ethanolic of *Portulaca oleracea* Linn. on the ethylene glycol and ammonium chloride-induced urolithiasis. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2013; 5(2): 134-140.
- Ula DQ, Azizah N, Suryanto A. Pembungaan kembali tanaman mawar (*Rosa Sp.*) sebagai tanaman taman melalui pemangkasan dan pemberian pupuk. *Plantropica Journal of Agricultural Science*. 2019; 4(1): 1-10.
- Hariana, HA. Tumbuhan obat dan khasiatnya seri 3. Cetakan 3. Penebar Swadaya. Jakarta. 2007.
- Boskabady MH, Shafei MN, Saberi, Amini S. Pharmacological effects of *Rosa damascena*. *Iranian Journal of Basic Medical Sciences*. 2011; 14(4): 295-307.
- Farnsworth NR Biological and phytochemical screening of plants. *Journal of Pharmaceutical Sciences*. 1966; 55(3): 225-276.
- Boskabady, Mohammad H, Mohammad NS, Zahra S, and Somayeh A. 2011. "Pharmacological Effects of *Rosa Damascena*." *Iranian Journal of Basic Medical Sciences* 14(4):295-307.
- Akram, Muhammad, Muhammad R, Naveed M, Naheed A, Sadia Z, Farhat J, Mohammad AS, Naheed A Zarfishan R, Syed HA, Muhammad D, Rabia Z, and Fahad SK. 2019. "Chemical Constituents, Experimental and Clinical Pharmacology of *Rosa Damascena*: A Literature Review." *Journal of Pharmacy and Pharmacology* 72(2):161-74.
- Achuthan, C. R., B. H. Babu, and Jose Padikkala. 2003. "Antioxidant and Hepatoprotective Effects of *Rosa Damascena*." *Pharmaceutical Biology* 41(5):357-61.
- Gholamhoseinian, A., H. Fallah, and F. Sharifi far. 2009. "Inhibitory Effect of Methanol Extract of *Rosa Damascena* Mill. Flowers on α -Glucosidase Activity and Postprandial Hyperglycemia in Normal and Diabetic Rats." *Phytomedicine* 16(10):935-41.

- Mahboubi, Mohaddese. 2016. "Rosa Damascena as Holy Ancient Herb with Novel Applications." *Journal of Traditional and Complementary Medicine* 6(1):10-16.
- Suherman, Chrismis NG, Linda C, Ali NN. 2021. " Nephroprotective Effect from Rose Petals (Rosa damascena) Extract Against Cadmium Toxicity." 2021 IEEE International Conference on Health, Instrumentation & Measurement, and Natural Sciences (InHeNce).
- Khaliq T, F Mumtaz, ZU Rahman, I Javed and A Iftikhar, 2015. The nephroprotective potential of Rosa damascena mill flowers, Cichorium intybus Linn roots, and their mixtures on gentamicin-induced toxicity in albino rabbits. *Pak Vet J*, 35(1): 43-47.
- Januar R, Wientarsih I, Widhyari SD. Uji kelarutan kristal kalsium oksalat urin tikus Sprague dawley setelah pemberian eliksir ekstrak etanol daun pelawan. *Jurnal Veteriner*. 2020; 21(1): 106-114.
- Verdes S, Fogazzi GB, Messa GPP, Daudon M. Crystalluria: prevalence, different types of crystals and the role of infrared spectroscopy. *Clin Chem Lab Med*. 2011; 49(3): 515-520.
- Steven L. Stockham, Michael A. Scott. 2008. *Fundamentals of Veterinary Clinical Pathology*. Blackwell Publishing. The USA.
- Narendra Nalwaya, E. Edwin Jerald, Suhail Asghar & Showkat Ahmad. 2009. Diuretic activity of a herbal product UNEX. *International Journal of Green Pharmacy*. 225-226.
- S. Bouanani, C. Henchiri, E. Migianu-Griffoni, N. Aouf, M. Leucovey. 2010. Pharmacological and toxicological effects of *Paronychia argentea* in experimental calcium oxalate nephrolithiasis in rats. *Journal of Ethnopharmacology*. 129: 38-45.
- Nancy A. Brunzel. 2013. *Fundamentals of urine and body fluid analysis*. Third edition. Elsevier Saunders. Missouri (US).
- Jie Fan, Michael A. Glass & Paramjit S. Chandhoke. 1999. Impact of ammonium chloride administration on a rat ethylene glycol urolithiasis model. *Scanning Microscopy*. 13(2-3) : 299-306.
- G.B. Fogazzi. 1996. Crystalluria: a neglected aspect of urinary sediment analysis. *Nephrol Dial Transplant*. 379-387.
- Touhami M, Laroubi A, Elhabazi K, Loubna F, Zrara I, Eljahiri Y, Oussama A, Grasses F, Chait A. 2007. Lemon juice has protective activity in a rat urolithiasis model. *BMC Urology*. 2007; 7:18.
- Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of *Acorus calamus* L. rhizome in experimental animal models. *Journal of Traditional and Complementary Medicine*. 2016; (30): 1-6.
- Cruzan G, Corley RA, Hard GC, Mertens JJWM, McMartin KE, Snellings WM, Gingell R, Deyo JA. Subchronic toxicity of ethylene glycol in Wistar and F-344 rats related to metabolism and clearance of metabolites. *Toxicological Sciences*. 2004; 81:502-551.
- Wientarsih I, Madyastuti R, Prasetyo BF, Aldobrata A. Anti lithiasis activity of avocado (*Persea Americana* Mill) leaves extract in white male rats. *Journal of Biosciences*. 2012; 19(1): 49-52.
- Dalton RN. Serum creatinine and glomerular filtration rate: perception and reality. *Clinical Chemistry*. 2010; 56(5): 687-689.
- Laksmi NLGMC, Dada IKA, Damriyasa, IM. The bioactivity of the tapakdara leaf extract (*Catharanthus roseus*) on creatinine and blood urea of rats (*Rattus novvergicus*). *Bulletin Veteriner Udayana* 2014; 6(2): 147-152.
- Suryawan DGA, Arjani IAMS, Sudarmanto IG. Gambaran kadar ureum dan kreatinin serum pada pasien gagal ginjal kronis yang menjalani terapi hemodialisis di RSUD Sanjiwani Gianyar. *Meditory*. 2016; 4(2): 145-153.
- Jouad H, Lacaille-Dubois MA, Lyoussi B, Eddouks M. Efficacy of the flavonoid extracted from *Spergularia purpurea* Pers. On the arterial blood pressure and renal function in normal and hypertensive rats. *Journal of Ethnopharmacology*. 2001; 76: 159-163.