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The effectiveness of avocado (*Persea americana* M.) leaves infusion as urinary stones dissolving in rats

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ABSTRACT

Urolithiasis is a pathological condition marked by the formation of urinary stones (calculi) in the urinary tract. Avocado leaf (*Persea americana* M.) contains substances that play crucial roles in the solubility of calcium in urinary stones, such as flavonoids, alkaloids, saponins, and magnesium. Empirically, avocado leaves are used to make urinating easier and break stones in the urinary tract. This study aims to determine the effect of avocado leaf infusion to dissolve urinary stones in white male rats. According to the experiment, 24 male white rats were divided into four groups; the negative control group (not given infusion), the 1 g/kg BW dose group, the 2 g/kg BW dose group, and the 3 g/kg BW dose group of avocado leaves infusion. The infusion was administered orally for seven consecutive days after 28 days of planting black silk thread as the stone core in the urinary tract. Then, the stone formed is taken. The weight and volume are measured. The urinary stones were tested qualitatively in terms of calcium and oxalate. The results showed that there were significant differences among treatment groups in weight and volume of urinary stones. The effect of bladder stone dissolving was best in the 3 g/kg BW group. The qualitative test also shows that urinary stones contain the same calcium and oxalate as urinary stones that form in humans. Avocado leaves infusion can have a urinary stone dissolving effect on white male rats. Increasing the dose of *avocado leaves* infusion increases its effectiveness as a urinary stone dissolution.

Keywords: avocado leaves, infusion, *Persea americana* M., urinary stones, urolithiasis

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INTRODUCTION

Urolithiasis is a pathological condition with the formation of urinary stones (calculi) throughout the urinary tract, which can be caused by impaired urine flow, metabolic disorders, urinary tract infections, dehydration, and other unexplained conditions (idiopathy). Urinary stones are made up of crystals in the urinary tract. The crystals are formed from organic and inorganic substances that dilute in the urine. Crystals attach to the epithelium of the urinary tract (formation of crystal retention), and other substances deposit on the aggregate, forming large stones that block the urinary tract. The urinary stone that has been known since the Babylonian and Ancient Egyptian times was found in a mummy's urinary system. This disease can attack people all over the world, including the population in Indonesia. The incidence of this disease is not the same in different parts of the world. Patients with urinary stones are found mostly in developing countries, while upper urinary tract stones are found mostly in developed countries. The occurrence is due to the influence of nutritional status and daily activities of patients (Purnomo, 2016). The data shows that the prevalence of the Indonesian population who suffer from urinary stones is 0.5% of the total population of Indonesia. Meanwhile, the recurrence rate is 50% within five years and 70% within ten years. Thus, it makes bladder stone disease requires special attention. Urinary stones contain a high calcium (calcium phosphate or calcium oxalate). 85% of patients with urinary tract stones are of this type, 10% are uric acid stones, and the rest contain various ingredients, namely cysteine, mineral phosphate, and struvite (Dewi & Subawa, 2007).

One of the causes of urinary stones is a lack of water intake (Purnomo, 2016). Water needs may vary widely among individuals. Age, gender, jobs, temperature, and humidity of the environment, as well as physical activities, can influence the needs of water intake. Adequate water consumption for adults in a basal state is 2 liters in 24 hours (Susanto et al., 2011). There are 37.3% of adolescents whose fluid intake is less than 90% of their needs or the risk of dehydration (Buanasita et al., 2015).

Currently, the treatment for bladder stone disease varies widely, such as by medical means, Extracorporeal Shock Wave Lithotripsy (ESWL), endourology, laparoscopic surgery, or open surgery. However, all of these treatments cost a lot of money, let alone recurrent urinary stones. Therefore, alternative treatments that have high effectiveness and are inexpensive are needed (Purnomo, 2016). One of the efforts is to use traditional medicine. Previous researchers have studied the decay of urinary tract stones, both in the kidneys and bladder. Some natural ingredients that have been studied have an effect as the decay of urinary stones (Dharma et al., 2014; Inayati, 1997; Indriyana, 1997; Roza, 1996; Wirmijatie, 1995). The plant empirically used as a laxative for urinary stones is the avocado plant (*Persea americana* M.). Avocado plants have previously been used as traditional medicine, especially its fruit and leaves. Avocado leaf (*Persea americana* M.) contains substances that play crucial roles in the solubility of calcium in urinary stones such as flavonoids, alkaloids, saponins, and magnesium. Avocado leaves are used to make urinating easier and break stones in the urinary tract (Nisa & Astana, 2018). Some studies reported that avocado leaves *in vitro* can inhibit the formation and sloughing of urinary stones (Kristianingsih & Wiyono, 2015; Madyastuti et al., 2015). This study was to determine the effect of avocado leaf infusion as a urinary stone laxative and to determine the effect of increasing the dose of avocado leaf infusion on its effectiveness as urinary stone decay in rats.

MATERIALS AND METHODS

Materials

The materials used were avocado leaf (*Persea americana* M.) obtained from the Research Institute for Spices and Medicinal Plants (BALITRO) Bogor, Indonesia and determined by Herbarium Depokensis (DEP), Biota Collection Room, Universitas Indonesia.

Methods

Preparation of avocado leaf infusion

The avocado leaves were collected. The leaves were then sorted and washed with running water to remove impurities that were still attached to the leaves. Furthermore, the avocado leaves were

chopped into small pieces to speed up the drying process. Twenty grams of simplicia avocado leaves were boiled using 100 ml water for 15 minutes, starting the temperature to reach 90°C while occasionally stirring. Strain using flannel cloth, added enough hot water through dregs until infusion volume of 100 ml was obtained.

The effect of urinary stone decay *in vivo* testing

This research was an experimental study conducted at the Pharmacology Laboratory of the Faculty of Pharmacy, Pancasila University, Srengseng Sawah, Jagakarsa, South Jakarta. This research was carried out after the Health Research Ethics Committee UPNVJ, in order to protect the rights and welfare of the health research subjects obtaining approval from the Universitas Pembangunan Nasional Veteran Jakarta (approval number: 186/VI/2021/KEPK). Wistar male white rats were acclimatized for one week in cages to adapt to the new environment. During acclimatization, rats were given food and water equally. The study was initiated by surgically implanting urinary stones in the form of lumps of black silk thread, as the core of the stone disease, into the bladders of rats. Rats were submitted to anesthesia with xylazine (Interchemie, Holland) and ketamine hydrochloride (Hospira, Inc.,USA) via intraperitoneal. The lower abdominal area was cleaned with 70% alcohol using a cotton swab then a skin incision was made in the lower abdomen (approximately 1 cm from the base of the scrotum). By using small test tweezers, the bladder was removed from the abdominal cavity. The bladder was cut open with small sharp scissors, and then the bladder was inserted with a thread loop then the wound was closed with one stitch. The bladder was then put back into the abdominal cavity. The surgical wound was sutured in 2 stages; muscle sutures peritoneum and external sutures to the skin. Rats were injected with antibiotics (Ceftriaxone, PT.Dankos Farma) intraperitoneally, and antiseptic was given to the wound. After the surgery, the rats were placed in clean cages. From day 2 to day 28, rats were fed and drank moderately (Inayati, 1997; Roza, 1996). From day 29 to day 35, rats have given avocado leaves infusion orally for seven days consecutively with three different doses. The doses are 1 g/kg BW, 2 g/kg BW, 3 g/kg BW. On day 36, the rats were euthanized, and the urinary stones formed were taken. The urinary stones taken were then dried in an oven at 40ºC to a constant weight, then the stones were weighed using an analytical balance, and the volume was measured. Qualitative tests were applied on the content of calcium and oxalate.

Weight and volume of urinary stones calculation

Urinary stones formed first were dried in an oven at 40º C to constant weight. Then, the stones' weight was measured using an analytical balance one by one. The urinary stone volume was obtained by using the formula of the ball volume = $\frac{4}{3} \times \pi \times r^3$ moderately (Inayati, 1997; Roza, 1996).

Qualitative analysis of urinary stones

The dried stone was grounded with a mortar and pestle until it became a powder. Calcium analysis was carried out by adding 3 mL of 10% HCl to the powder, then heated and added saturated NH4 oxalate through the tube wall (do not shake). If it contains calcium, it will form a precipitate. Oxalate analysis was carried out by adding 1 mL of 10% HCl to the powder, bringing it to a boil, and adding a spoonful of MnO2. If it contains oxalate, gas will appear. The results of the qualitative assessment are shown with (+++) lot of precipitation and gas formed, (++) medium precipitation and gas formed, (+) less precipitation and gas formed (Kristianingsih & Wiyono, 2015).

Data Analysis

The data were analyzed using SPSS (Statistical Package for the Social Science) application. The data are tested in terms of normality (Kolmogorov-Smirnov test) and homogeneity (Levene test). After the data are normally distributed and homogeneously varied, parametric statistical tests are performed using the one-way analysis of variance (ANOVA) method, if the results showed significant

differences, followed by Post Hoc LSD (Least Square Differences) test to see meaningful differences in each treatment group and then the Mann-Whitney test to observe the difference in each treatment group**.**

RESULT AND DISCUSSION

In this study, the results are in the form of urinary stones in each treatment group taken on day 36 in rats. From the result of the study, the negative control group not given avocado leaf infusion showed large urinary stone formation. The group dose of 1g/kg BW showed a decrease in macroscopic appearance when compared to the negative control group. In the group given an avocado leaf infusion dose of 2 g/kg BW, there is a more reduction in macroscopic appearance. Meanwhile, the group at a dose of 3 g/kg BW shows the most decreased macroscopic appearance (Figure 1. (A-D)).

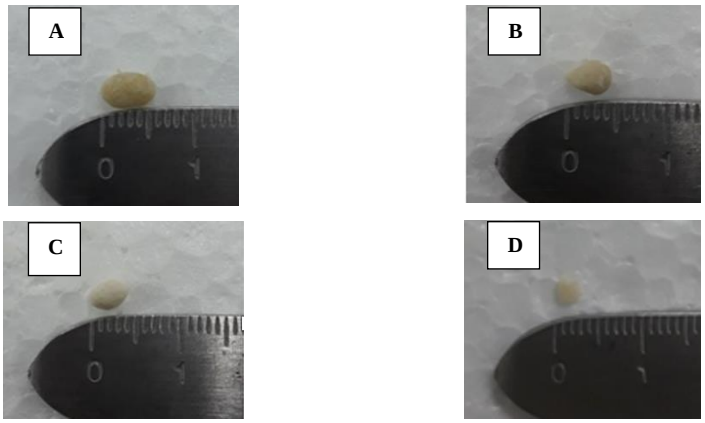

Figure 1. Representative macroscopic appearance of the urinary stone in rats. (A) Negative group; (B) Dose of 1 g/kg BW group; (C) Dose of 2 g/kg BW group; and (D) Dose of 3 g/kg BW group

In this *in vivo* study, white male rats of the Wistar strain are manipulated to have urinary stones by planting black silk threads that are intended as triggers for the formation of stones in the rat's bladder. Urinary stones are successfully made in this study. It is indicated by the negative control group, which is not given avocado leaf infusion. The results show an average urinary stone weight of 86.32 mg and an average urinary stone volume of 0.07 cm^3. The previous study also showed that black silk thread implanted in the urinary tract could trigger the formation of urinary stones with a size of 1 cm or ± 5 knots (Inayati, 1997; Roza, 1996). This study shows that animal models suffering from urinary stones can be used to test urinary stone dissolving drugs.

In this study, avocado leaf infusion was administered at three different doses; a dose of 1 g/kg BW, 2 g/kg BW, and 3 g/kg BW. The negative control group, not given avocado leaf infusion, showed the formation of urinary stones with the highest weight occurs. The group of 1 g/kg BW showed a decrease in the weight and volume of urinary stones when compared to the negative control group. In the group dose of 2 g/kg BW, there is a more reduction in the weight of urinary stones. Meanwhile, the group dose of 3 g/kg BW shows the most decrease in urinary stone weight. The results of weighing urinary stones can be seen in Figure 2.

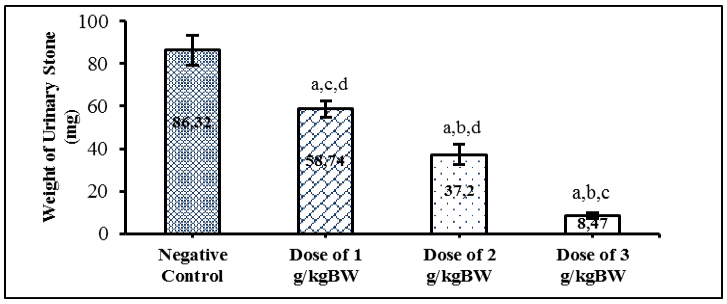

Figure 2. Decreased weight of urinary stones (mg) after given avocado leaves infusion orally for 7 days. Data are presented in mean±SD and obtained from 4 separate groups. a) *p-value* <0.05 vs negative group, b) *p-value* <0.05 vs group dose of 1 g/kgBW, c) *p-value* <0.05 vs group dose of 2 g/kgBW, d) *p-value* <0.05 vs group dose of 3 g/kgBW. a, b, c, d *p-value* <0.05 after performing *one-way ANOVA* test followed by *Post Hoc LSD*

This study also measured the volume of urinary stones. The negative control group not given avocado leaf infusion showed the formation of urinary stones with the highest volume occurs. The group dose of 1 g/kg BW showed a decrease in the volume of urinary stones when compared to the negative control group. In the group dose of 2 g/kg BW, there is a more reduction in the volume of urinary stones compared to the 1 g/kg BW dose group and negative control. Meanwhile, the group dose of 3 g/kg BW shows the most decrease in urinary stone volume compared to the 2 g/kg BW, 1 g/kg BW, and negative control group. The results of the calculation of urinary stone volume can be seen in Figure 3.

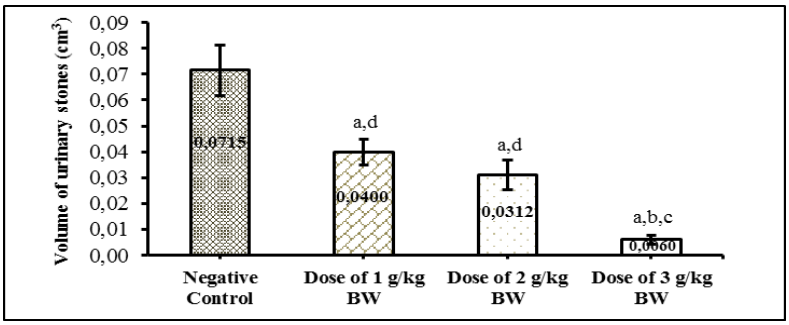

Figure 3. Decreased volume of urinary stones (mg) after given avocado leaves infusion orally for 7 days. Data are presented in mean±SD and obtained from 4 separate groups. a) *p-value* <0.05 vs negative group, b) *p-value* <0.05 vs group dose of 1 g/kgBW, c) *p-value* <0.05 vs group dose of 2 g/kgBW, d) *p-value* <0.05 vs group dose of 3 g/kgBW. a, b, c, d *p-value* <0.05 after performing *one-way ANOVA* test followed by *Post Hoc LSD*

From the result of the study, it can be seen that the weight and volume of urinary stones formed in each dose group, there is a significant decrease in both the weight and volume of urinary stones formed. Thus, there is a dissolving effect on urinary stones, as well as there is an effect of adding the dose on improving the effectiveness of dissolving urinary stones in Male white rats. The avocado leaf contains flavonoid and magnesium compounds that can dissolve urinary stones (Kristianingsih & Wiyono, 2015). Research on anti-urolithiasis has been widely studied from the 1980s to the present. Previous research on infusion leaves of some plants that contain flavonoid compounds that are also able to dissolve urinary stones (Anas et al., 2014; Dharma et al., 2014; Hutabalian, 2018; Lubis, 2018; Novitri et al., 2018).

Urinary stones are made up of crystals in the urinary tract. The crystals are formed from organic and inorganic substances that dilute in the urine. These crystals remain metastable (dissolved) in urine unless there are specific conditions that cause the crystals to precipitate. The crystals precipitate each other to form a rock core (nucleation), aggregate, and attract other materials. And then they become larger crystals. Even though they are quite large, the crystal aggregates are still fragile and not sufficiently capable of clogging the urinary tract. As a result, crystal aggregation adheres to the urinary tract epithelium. From there, other materials are stored on top to form larger stones to obstruct the urinary tract. The metastable state is influenced by temperature, pH of the solution, presence of colloids in urine, the concentration of urinary solutes, amount of urine in the urinary tract, or presence of a corpus alienum in the urinary tract acted as the core of the stone. More than 80% of urinary stones are made up of calcium stones, both of which bind with oxalates and phosphates to form calcium oxalate and calcium phosphate stones, while the rest comes from uric acid stones, infectious stones, xanthine stones, cysteine stones, and other stones. Although the pathogenesis of stone formation is nearly the same, the environment in the urinary tract that allows the formation of these stones is different. For example, in this case, magnesium and ammonium phosphate stones are formed because urine is alkaline meanwhile uric acid stones are formed easily in an acidic environment (Purnomo, 2016).

Whether or not stones are formed in the urinary tract is also determined by the balance between inhibitory stone-forming substances, which are substances that can prevent stones from forming. There are known substances that can inhibit the formation of urinary tract stones, which work starting from the calcium reabsorption process in the intestine, the process of forming a stone or crystal core, the process of crystal aggregation, to crystal retention. Magnesium ion (Mg++) is known to inhibit stone formation because if it binds to oxalate, it forms magnesium oxalate salt so that the amount of oxalate that will bind with calcium (Ca++) to form calcium oxalate decreases. Likewise, if citrate binds with calcium ions (Ca++) it forms a calcium citrate salt, so the amount of calcium that will bind to oxalate or calcium phosphate is reduced (Purnomo, 2016). Urinary stones have various types such as calcium oxalate stones, calcium phosphate, magnesium ammonium phosphate, xanthine, cysteine, and uric acid (Lotan, 2012; Stoler, 2008).

In this study, urinary stones formed are tested for qualitative analysis of the calcium and oxalate content. For qualitative analysis of calcium, it contains calcium if it would form a precipitate, while oxalate; contains oxalate if it would produce gases. From the results of the qualitative test, it was found that urinary stones, especially in the negative control group, contained high amounts of calcium and oxalate, which was indicated by the formation of precipitation in calcium analysis, and gas appeared in oxalate analysis. In the 1 g/kg BW dose group, there is a decrease in the number of precipitation and gas compared to the negative group. In the group of 2 g/kg BW and 3 g/kg BW doses, there is a small amount of precipitate in the analysis of calcium and small gas content in the analysis of oxalate content. The qualitative test of calcium and oxalate contents results can be seen in Table 1, and representative precipitation can be seen in Figure 4.

Table 1. Qualitative test data for calcium and oxalate content

Group	Calcium	Oxalate
Negative Control	+++	+++
Dose of 1 g/kg BW	++	++
Dose of 2 g/kg BW	+	+
Dose of 3 g/kg BW	+	+

(+) = presence of constituents

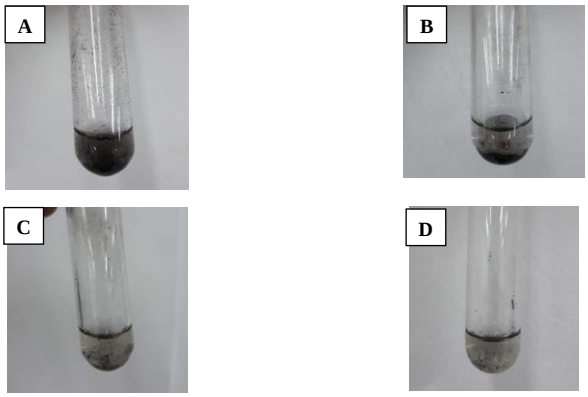

Figure 4. Representative precipitation appearance of urinary stone in calcium analysis. (A) Negative group; (B) Dose of 1 g/kg BW group; (C) Dose of 2 g/kg BW group; and (D) Dose of 3 g/kg BW group

From the results of qualitative tests, especially in the negative control group, which had relatively larger stone sizes compared to the 1 g/kg BW, medium, and high dose groups, it is stated that urinary stones in the negative control group contained calcium and oxalate. Urinary stones in the 1 g/kg BW, medium, and high doses also contain calcium and oxalate but are not as large as the negative control group. With the presence of calcium and oxalate in the urinary stones formed, it can be stated that urinary stones in rat models have the same formation as urinary stones in humans. Substances that play an important role in the calcium solubility process in urinary stones such as flavonoid compounds, alkaloids, saponins, magnesium and potassium from the extract (Winarti et al., 2014). Factors that play a role in dissolving kidney stones are due to the presence of secondary metabolites such as flavonoids and these alkaloids (Nessa et al., 2013). Avocado leaf infusion containing flavonoids, alkaloids, saponins, kalium, and magnesium can dissolve calcium and reduce the size of urinary stones. Avocado plant (*Persea americana* M.) is used as traditional medicine, especially the fruit and leaves (Kristianingsih & Wiyono, 2015). Avocados can empirically be used for urinary stones, high blood pressure, lowering high cholesterol levels, back pain, headaches, nerve pain, stomach pain, swollen respiratory tract, back pain, stomach pain, dysentery, irregular menstruation, gallstones, and diabetes mellitus (Syamsuhidayat & Hutapea, 1991).

Flavonoids are a large group of polyphenols that consist in plants. Flavonoids have advantageous effects against a number of common diseases. The results of recent studies have shown that flavonoids can effectively inhibit calcium oxalate (CaOx) stone formation *in vitro* and *in vivo*, correlating with a diuretic, antioxidant, anti-inflammatory effects, antibacterial, anti-infective, anti-apoptotic, protective of the microcirculation, which regulate the synthesis and expression of endogenous stone activators or inhibitors, maintain the balance of oxalate metabolism or reduce crystal size and number, and other protective effects (Zeng et al., 2019). As an anti-inflammatory and antioxidant compound, flavonoids can interfere with CaOx crystal-induced epithelial cell damage and inhibit inflammation (Thamilselvan et al., 2003; Yuen et al., 2010). The diuretic and antibacterial effects of the extracts should not be overlooked because the role of bacterial origins, such as nanobacteria, is equally important in the formation of CaOx crystals (Izzo & Ernst, 2009; Saddiqe et al., 2010).

Mucoproteins have a high affinity for CaOx surfaces to promote crystal growth and deposition. The extract that is rich in flavonoids can dissolve CaOx kidney stones through the degradation of mucoproteins (Leal & Finlayson, 1977). Flavonoids reduce oxidative stress via antioxidant activity and reduce cell damage via inflammatory activity, maintaining the integrity of the membrane and increasing the expression of crystal inhibitors in the kidney (Zeng et al., 2019).

CONCLUSION

Avocado leaf infusion (*Persea americana* M.) could have the effect of dissolving urinary stones in rats, and increasing the dose of avocado leaves infusion increases its effectiveness as a bladder stone dissolving.

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