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Research Article

ANALYSIS ON CALCIUM SOLUBILITY IN KIDNEY STONES (*IN VITRO*) AND DIURETIC EFFECT (*IN VIVO*) USING CORN SILK (*ZEA MAYS* L.) INFUSE

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ABSTRACT

Objective: Investigation has been carried out about infuse of corn silk (*Zea mays* L.) concerning the solubility of kidney stone *in vitro* and the diuretic effect *in vivo.*

Methods: The methodology of research conducted in the form of dissolving kidney stones in corn silk infuse with a concentration of 2%, 4%, 6%, 8%, and 10%, then incubated at 37°C for 4 h. The solubility of calcium is known by measuring the levels of early calcium and after incubated with kidney stones. Potassium (K) is a substance that can dissolve kidney stones in infuse and also measured by atomic absorption spectrophotometry. The diuretic effect study used male rats which were divided into 7 groups that consisted of 1 positive control (treated with furosemide), 1 negative control treated with CMC Na 0.5 % and 5 groups treated with infuse of corn silk in 5 different concentration (2%, 4%, 6%, 8%, and 10%).

Results: Increase in calcium levels after incubation with kidney stone using corn silk infuse with various concentrations. The levels of Ca dissolved in the infuse of 2%, 4%, 6%, 8%, and 10%, respectively, were 2.2600 µg/ml, 5.5733 µg/ml, 7.9267 µg/ml, 10.9233 µg/ml, and 8.7667 µg/ml. It was found that the urine volume for 24 h was significant with 2%, 8%, and 10% corn silk infuse.

Conclusion: Infuse of corn silk could decrease kidney stones, and it was diuretic. This research supported the aim of traditional corn silk decoction for the same intention.

Keywords: Solubility, Kidney stones, Male rats, Corn silk infuse, Diuretic, Atomic absorption spectrophotometry.

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INTRODUCTION

Indonesia inherits various traditional medicines with abundant herbs. However, since Indonesia consists of a lot of islands inhabited by various tribes, it might bring about the differences in the use of plants as the traditional medicines. This is due to each tribe had specific empirical and unique cultural experience accordance with their respective region [1]. Some medicinal plants mostly used traditionally by people to forestall and to shed kidney stones and to urinate smoothly are *Orthosiphon stamineus* L, *Sonchus arvensis* L., *Phyllanthus niruri* L., and *Zea mays* L.

Kidney stones frequently occur in the tropical countries. Tropical weather causes dehydration which will eventually cause the thickening of urine and becomes stone. Kidney stones are stones which are found in kidney and in urinary tract [2].

Some medicinal plants mostly used traditionally by people to forestall and to shed kidney stones and to urinate smoothly are *O. stamineus* L, *S. arvensis* L., *P. niruri* L., and *Zea mays* L.

One of these plants which are used by the people as traditional medicine is corn. A part of corn which can be used to cure urological disorder and play an important role as a diuretic agent in corn silk which is usually made in the form of *dekok* [3]. High content of potassium can destroy calcium salt in kidney stones because potassium will get rid of calcium to join with the carbonate, oxalate, and phosphate, or uric forming potassium oxalate, potassium carbonate, potassium phosphate, or potassium urate compounds that soluble in water [4].

Several researchers had done their researches on some plants which could dissolve kidney stones. For example, the study about ethanol extract of corn silk has the ability to dissolve kidney stones of calcium and measured by atomic absorption spectrophotometer. According to Hidayati, *et. al*., points out that the use of *tempuyung* dried tea leaves with certain frequencies can dissolve calcium oxalate as measured using gravimetric method [5,6]. Based on these researches, it was found that the compound of potassium and flavonoids involve in the decaying of kidney stone. In this research, the test decaying of kidney stone would be done by measuring the calcium dissolved in corn silk infuse using Atomic Absorption Spectrophotometry and diuretic effect test from corn silk infuse *(in vitro)* in male white mice.

METHODS

Apparatus

The tools used in this study were glassware, hot plate (Fisons), incubator, pH paper, filter paper, Whatman No. 42 filter paper, analytical balance (Ohaus), scale for animal, microscope (Nikon), infusing pot, volumetric flask, spatula, oral needle, mouse cage, a set of Atomic Absorption Spectrophotometer (Hitachi Z 2000) with acetylene-air flame, completed with calcium and potassium cathode lamp, and thermometer.

Materials

The materials used in this study were dry corn silk of Zea mays plants, kidney stones obtained from kidney stone patients at Firdaus Village, Sei Rampah Subdistrict, Serdang Bedagai Regency, furosemide, aqua demineralized and quality materials E. Merck pro analysis of CMC, nitrate acid of 65% v/v, potassium standard solution of 1.000 µg/ml, calcium standard solution of 1.000 µg/ml, and lanthanum oxide.

Guinea pig

Guinea pig consisted of 30 male white mice that age was about of 3 months and weight was about of from 150 g to 200 g.

Preparation of corn silk of *Zea Mays* infusion

The fresh corn silk is cleaned from the impurities and then washed with running water and drained, until dry. After the sample was dried, it was

cut into thin or small slices. After that, the infuse is made according to the procedure of Indonesian Pharmacopoeia, 4rd, by weighing in 10, 20, 30, 40, and 50 g; then put into infuse pot, and filled it with aqua demineralized up to 500 ml to each various concentration of 2%, 4%, 6%, 8%, and 10% is heated at a temperature of 90°C for 15 min while occasionally stirring, squeeze while hot through flannel, added more aqua demineralized though the dregs to obtain the volume of infuse was 500 ml [8].

Assay of kidney stone solubility *(in vitro)*

Kidney stone preparation

Kidney stones obtained from kidney stone patients at Firdaus Village, Sei Rampah Subdistrict, Serdang Bedagai Regency, were cleaned up with demineralized water, drained and aired until they dried up.

Making calibration curve of calcium and potassium

a. Measured each absorbance of the standard solution of potassium 2.0, 4.0, 6.0, 8.0, and 10 µg/ml with atomic absorption spectrophotometer at a wavelength of 766.5 nm, respectively.
b. Measured each absorbance of the standard solution of calcium 2.0, 4.0, 6.0, 8.0, and 10 µg/ml with atomic absorption spectrophotometer at a wavelength of 422.7 nm, respectively.

Preparation infuse and assay for initial calcium and potassium

About 50 mL each concentration of infuse solution using a flask, put it into 150 mL erlenmeyer flask, for initial calcium and potassium analysis. Diluted. An absorbance of the solution is measured by atomic absorption spectrophotometer at a wavelength of 766.5 nm for potassium and 422.7 nm for calcium with the type of air-acetylene flame. Absorbance values obtained should be within the range of the calibration curve of a standard solution of potassium and calcium.The concentration of potassium and calcium in the sample is calculated based on the regression equation of the calibration curve.

PREPARATION INFUSE AND ASSAY OF CALCIUM AFTER INCUBATION WITH KIDNEY STONE

About 50 mL each concentration of infuse solution was then put into 150 mL Erlenmeyer flask, included kidney stones and incubation at 37°C for 4 h and stirred every 10 min. Analysis of calcium levels after incubation with kidney stones in the same handling with the analysis of the initial calcium levels.

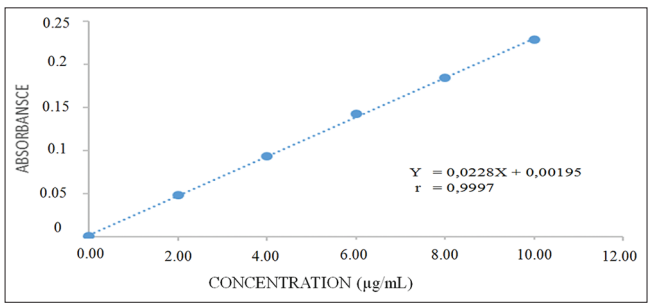

Fig. 1: Calcium calibration curve

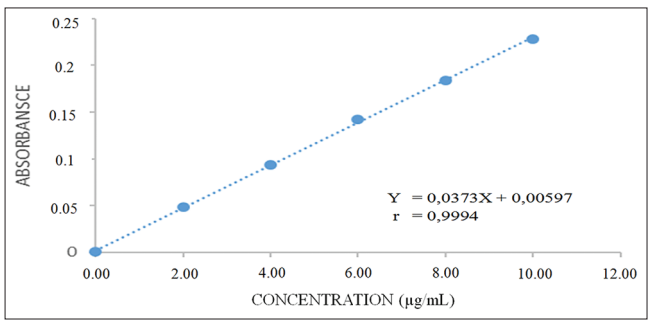

Fig. 2: Potassium calibration curve

Diuretic effect assay of infuse (*in vitro*)

The guinea pig used male white mice with the weight of 150–200 g and was 2–3 months old. The mice were acclimatized in about 1 week.

Dosage

The dosage used for the testing of diuretic effect in corn silk infuse of 2%, 4%, 6%, 8%, and 10% was for 300 mg/kg BB, 600 mg/kg BB, 900 mg/kg BB, 1200 mg/kg BB, and 1500 mg/kg BB, respectively.

Treatment on guinea pig

The guinea pig was grouped into 7 (seven) groups which consisted of 5 (five) treatment group, 1 (one) positive control group, and 1 (one) negative control group in which each group consisted of 4 (four) mice. Furosemide with the dosage of 3.6 mg/kg BB was orally given to the positive control group, and CMC Na, 0.5% solution, was orally given to the negative control group. In the treatment group, the preparation of 2% of corn silk infuse was given to Group I which consisted of 4 (four) mice with 2.185 ml, 2.325 ml, 2.370 ml, and 2.400 ml, respectively; 4% of infuse was given to Group II which consisted of 4 (four) mice with 2.406 ml, 2.475 ml, and 2.541 ml, respectively; 6% of infuse was given to Group III with 2.77 ml, 2.85 ml, 2.88 ml, and 2.92 ml, respectively; 8% of infuse was given to Group IV with 2.625 ml, 2.639 ml, 2.645 ml, and 2.670 ml, respectively; 10% of infuse was given to Group V with 2.728 ml, 2,729 ml, 2,742 ml, 2,756 ml, and 2,775 ml, respectively. Measurement in the 1st h, 5th h, and 24th h was done to test the volume of urine within 24 h.

RESULTS AND DISCUSSION

Calcium and potassium calibration curve

The result of the measurement of calibration curve for calcium can be seen at Figs. 1 and 2, there was the equation of regression line: Y=0.0228X+0.00195 with coefficient correlation (r) of 0.9997.

The result of the measurement of calibration curve for potassium was the equation of regression line: Y=0.037344286X+0.005971 with coefficient correlation (r) of 0.9994.

Initial calcium and potassium content in corn silk infuse in various concentrations

The result could be seen in Table 1.

Table 1 shows that there is a difference between the average levels of calcium and potassium at a various concentration of infuse. The higher the concentration, the higher the levels of calcium and potassium in the infusion.

Dissolved Ca of kidney stone in infuse

Infusion solution in which kidney stones had been immersed in 4 h at the temperature of 37°C has measured with AAS 6 (six) times replication, then calculated the average and standard deviation (SD) value; the result could be seen in Table 2.

Dissolved Ca of kidney stone in each infuse concentration could be calculated and shown in Table 3.

Based on the data above, can be seen that there is the difference between initial calcium and calcium after incubation with kidney stones. This shows that corn silk infuse at various concentrations can dissolved

Table 1: Initial calcium and potassium content in various concentration of corn silk infuse

Concentration corn silk infuse (%)	Initial Ca content	Initial K content
2	10.8733	207.5000
4	16.3067	364.8333
6	18.2333	583.3333
8	20.9667	709.5000
10	24.3600	807.1667

Table 2: Calcium level in various concentration of corn silk infusion after incubation

Concentration corn silk infuse (%)	Average absorbance	Average concentration	Average Ca conc in infuse (µg/ml)
2	0.077	3.283	13.133
4	0.127	5.470	21.880
6	0.151	6.520	26.107
8	0.175	7.597	30.387
10	0.191	8.282	33.127

Table 3: Dissolved calcium in various concentration of corn silk infuse

Infuse concentration (%)	Initial K content (µg/ml)	Initial Ca content (µg/ml)	Ca after incubation (µg/ml)	Dissolved Ca content (µg/ml)
2	207.5000	10.8733	13.1333	2.2600
4	364.8333	16.3067	21.8800	5.5733
6	583.3333	18.2333	26.1600	7.9267
8	709.5000	20.9667	31.8900	10.9233
10	807.1667	24.3600	33.1267	8.7667

kidney stone, and can be concluded that there is no linear correlation between the amount of potassium in the infusion with the amount of dissolved calcium. This is most likely because the factor of the calcium salt that is not homogenous in the lining of the kidney stones, both in the type and number of number of compounds, so that the solubility of calcium salts concentration infuse that was made ascending, the increase is not linear. It could be described in the graph in Fig. 3.

Infuse solubility

According to Winarto and Tim Karyasari, potassium which makes kidney stones in the form of calcium salt is scattered, because potassium will get rid of the calcium contained as a compound of calcium oxalate, carbonate, phosphate, or uric which is forming kidney stones, and will form compounds of potassium salt which is more soluble in water, so the calcium salt in the kidney stones will dissolve slowly and come out with urine to be dissolved because potassium would change calcium as oxalate calcium compound, phosphate carbonate [7].

Levels of dissolved calcium are the increment calcium levels after incubated with kidney stones in corn silk infuse at 37°C for 4 h. Levels of dissolved Ca is the Ca levels after incubated minus the initial Ca levels. The ability of dissolving potassium ion toward calcium salt in the kidney stones caused by the position of potassium in the series volta is located in the left so that potassium will get rid of calcium to join carbonate, oxalate, phosphate, or urate, and calcium become soluble [7].

Validation od AAS method

Based on the data of calcium and potassium calibration curves, it was found there were detection limit and quantitation limit for calcium 0.4178 µg/ml and 1.3926 µg/ml. Detection limit and quantitation limit for potassium were 0.2506 µg/ml and 0.8325 µg/ml. From the results, can be seen that all results obtained in the samples above the limit of detection and quantitation limits.

Accuracy test

The result of accuracy test with recovery percentage parameter of calcium content after the addition of calcium standard solubility in the samples was obtained 102.24%. The recovery percentage indicated the accuracy of work during the examination of calcium content. The result of the Recovery Test had met the requirement for accuracy which had been stipulated - the average result of recovery was in the range of 80%–120% [9–11].

Relative SD (RSD)

From the results perfored on the measured data of the minerals calcium and potassium levels in phyllanthus niruri infuse, the value of relative standard deviation (RSD) is obtained at 1.2210% for the calcium mineral.

Based on Table 10, it can be seen the value of SD for calcium mineral is 1.4229 while the value of RSD is obtained at 1.2210% for the calcium mineral.

According to Harmita, the value of relative of RSD for analytes with levels of parts per million (µg/mL) is not more than 16%, and for analytes, with levels of parts per billion, its RSD is not more than 32%. From the results obtained indicate that the methods do have good precision [9].

Diuretic effect test on the guinea pig *in vivo*

The result of infuse diuretic effect test could be seen in Table 4.

From Table 4 , which was organized according to the observation and the collection of urine in 24 h, it was found that in 1 h observation and the collection, furosemide group had the highest urine volume (0.85±0.19 ml) and was significant on CMC Na 0.5% group ($p<0.05$), while in corn silk infuse group various concentrations did not have urine volume which was statistically significant difference ($p>0.05$) from the urine volume of CMC Na 0.5% group. In 5 h observation, furosemide group became the group with the highest urine volume (3.125±1.13 ml) and was significant on CMC Na 0.5% group ($p<0.05$), while in corn silk infuse group various concentrations did not have urine volume which was statistically significant difference ($p>0.05$) from the urine volume of CMC Na 0.5% group. In 24 h observation, urine volume groups which had significant difference ($p<0.05$) from the urine volume of CMC Na 0.5% group were furosemide group (8.075±0.65 ml), corn silk infuse 2% (6.9±0.61 ml), corn silk infuse 8% (6.9±0.6 ml), and corn silk infuse 10% (11.5±1.68 ml).

Based on the result of the observation, it was found that furosemide had diuretic activity with the highest urine volume in 1 h and 5 h observations. In 24 h observation, furosemide group, corn silk infuse of 2%, 8%, and 10% had diuretic activity as it could be seen in Fig. 4.

Corn silk infuse had bigger diuretic activity than furosemide, but it worked at a slow pace (24 h). Corn silk infusion had higher diuretic activity in 24 h test (subacute) than that in 5 h test (acute) [12]. The compound which plays the role as diuretic agent was flavonoid and the amount of potassium. The amount of potassium increased in the blood which caused potassium concentration in tubules also increased. This would bring about the increase in osmosis pressure in distal tubules and collectivist tubules. Osmosis law states that water will move from low concentration to high concentration so that high osmosis pressure in tubules would bring about water accumulation, and water would be excreted as urine and caused the incidence of the increase in urine production [5].

CONCLUSION

The solubility of calcium salt kidney stone corn silk infuse is 2.2600 µg/ ml solution in infuse of 2%, 5.5773 µg/ml in infuse of 4%, 7.9267 µg/ml in infuse of 6%, 10.9223 µg/ml in infuse of 8%, and 8.7667 µg/ml in infuse of 10%. It could be concluded that the higher the concentration of infuse, the higher the calcium solubility of kidney stones in infuse. Corn silk infuse

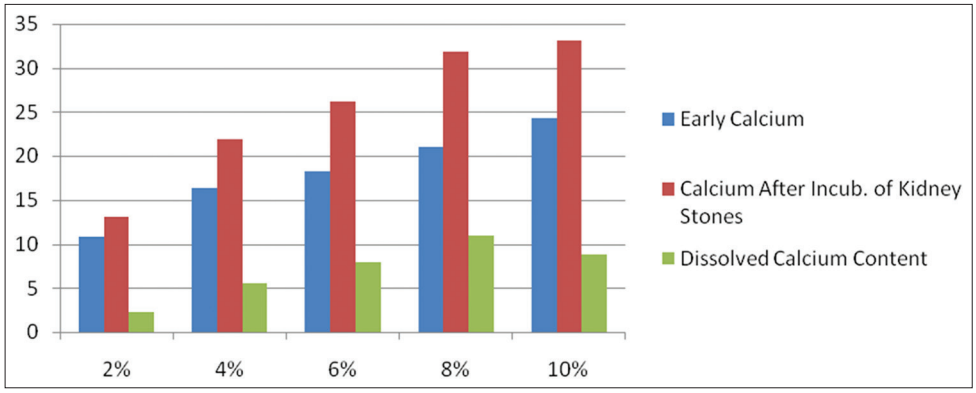

Fig. 3: Graph of dissolved calcium content in various concentrations of infusion

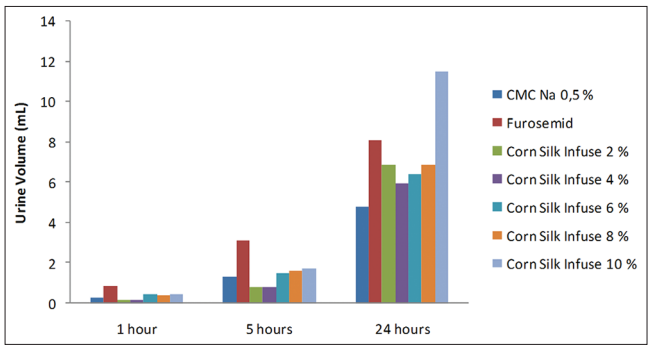

Fig. 4: Graph of diuretic activity

also had diuretic effect on the guinea pig, male white mice, but it worked very slowly (in 24 h). The result of this research supported the aim of the traditional use of corn silk stew as the shedding of kidney stones and as urine diuretics.

Table 4: Observation and measurement of urine volume of the mice

Group (%)	Average urine volume (ml)±SD		
	1 h	5 h	24 h
CMC Na 0.5	0.275±0.22[b]	1.35±0.34[b]	4.8±0.81[b]
Furosemide	0.85±0.19[a]	3.125±1.13[a]	8.075±0.65[a]
Corn silk infuse 2	0.175±0.23[b]	0.825±0.54[b]	6.9±0.61[a]
Corn silk infuse 4	0.175±0.23[b]	0.825±0.36[b]	5.9375±1.23[b]
Corn silk infuse 6	0.45±0.52[b]	1.5±1.13[b]	6.425±2.16[b]
Corn silk infuse 8	0.3875±0.44[b]	1.625±0.62[b]	6.9±0.61[a]
Corn silk infuse 10	0.45±0.42[b]	1.75±0.71[b]	11.5±1.68[a,b]

[a]p<0.05 from CMC Na 0.5% group (Negative Control), [b]p<0.05 from Furosemide group (Positive Control). SD: Standard deviation

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