Activity of A Nanoparticles Preparation Containing Watermelon Peel Extract and Kepok Banana Peel on Ureum and Creatinine Levels in Urolitogenesis Mice

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Abstract. Kidney stones are one of the causes of chronic kidney failure that can cause long-term complications. Kepok banana peel and yellow watermelon peel are still rarely used as medicine. The high flavonoid and potassium content in kepok banana and yellow watermelon peel can function as a kidney stone remover and improve kidney function. The preparation started with the extraction of kepok banana peel and yellow watermelon peel. Then, the flavonoid and potassium contents of the extracts were tested. Nanoemulsions were prepared by homogenization method at 15,200 rpm. Finally, the prepared nanoemulsion formula was evaluated. Nanoemulsion of kepok banana peel and yellow watermelon extracts had a size of 246.9 nm and a PDI of 0.618. The nanoemulsion also contained flavonoids and potassium with a pH value of 7 and a viscosity value of 2.70 cP. Nanoemulsion of kepok banana peel and yellow watermelon peel extracts can also reduce creatinine levels and there is no decrease in ureum levels in kidney stone model rats. The administration of nanoemulsion of kepok banana peel and yellow watermelon peel extracts did not significantly affect the decrease in ureum levels, but decreased creatinine levels in the nanoemulsion and nonnanoemulsion intervention groups. Keywords: nanoemulsion extracts, kepok banana peel, yellow watermelon peel, kidney stones, kidney function

1 Introduction

Kidney stones are one of the causes of chronic kidney failure; crystals that deposit in the kidneys cause a decrease in the amount of GFR and ESRD at a young age [1]. As much 1,499,400 people in Indonesia suffer from kidney stones, one of the most common kidney diseases in Indonesia, with a prevalence of 10% in women and 15% in men, according to data from Basic Health Research [2]. Kidney stones can also cause various long-term and acute complications. Kidney stones can also cause long-term complications such as strictures, obstruction, hydronephritis, and end in kidney failure. Treatment for kidney stones requires a lot of money because it requires hemodialysis therapy, kidney transplantation, surgery and chemical drugs [3]. Kidney stone treatment includes pharmacological and non-pharmacological treatment. Currently, many non-pharmacological therapies are being developed, including herbal medicine. Therapy with natural ingredients has the advantage of being inexpensive and producing the best results[4]. Natural materials that can be used are plants. Plants function as living chemical factories for the biosynthesis of the majority of secondary metabolites. Pharmaceutical drugs Commercial and herbal medicines derived from plants are derived from these metabolites [5]. Secondary metabolites found in natural ingredients include potassium and flavonoids. Natural ingredients that are thought to contain both have the ability to dissolve kidney stones . Examples of these natural ingredients are cat's whiskers, keji beling, tempuyung leaves, soursop leaves, moringa leaves, gotu kola leaves, kepok banana peels, and yellow watermelon peels[4][6] [7].

The largest group of phenolic compounds that can be found in nature are flavonoids. Flavonoids work by forming complex compounds between calcium in kidney stones and the -OH group of flavonoids, which ends in the formation of Ca-flavonoids[8]. This flavonoid complex compound is more soluble in water, helping urine to dissolve[9]. Potassium can help facilitate the excretion of urine by breaking down kidney stones into calcium oxalate. Potassium removes calcium and combines with the calcium oxalate compound, which then forms a salt compound that is easily soluble in water. Kidney stones will gradually dissolve and then be excreted with urine[10]. Secondary metabolites derived from natural ingredients such as kepok banana peels and yellow watermelon peels are still not widely accepted in society, especially in terms of treatment[11][12]. Industrial players do not take advantage of this waste management, which results in a large accumulation of waste[13].

The amount of waste from watermelon and Kepok banana are very high because consumption continues to increase. Banana consumption in Indonesia increased by

6,205 kilograms per capita in 2014 to 59,912 kilograms per capita in 2018. Watermelon consumption also increased by 6.14% between 2014 and 2018, resulting in an increase in watermelon rind waste[14][15].

The problem with using natural ingredients is that the doses that must be taken are very large and absorption is often hampered, this is because herbs, extracts and infusions are still used as preparations. Better treatment solutions can come from nanoemulsions with smaller particle sizes than those commonly used[16]. The greater surface area and free energy, nanosized materials can prevent sedimentation, flocculation, creaming, and coalescence, which are common causes of emulsion breakdown. Researchers hope that the use of nanoemulsion preparations will produce better absorption and solubility properties[17]. Research objectives (i) To determine the effect of kepok banana peel and yellow watermelon peel nanoemulsion and non-nanoemulsion in reducing urea levels based on in vivo methods. (ii) To determine the effect of kepok banana peel and yellow watermelon peel nanoemulsion and non-nanoemulsion in reducing creatinine levels based on in vivo methods.

2 Method

2.1.Research design

This research used a laboratory experimental design consisting of making a nanoemulsion of kapok banana peel extract and yellow watermelon peel as well as in vitro testing related to the dissolution of kidney stones[18].

This research was conducted at the UMY Phytomedicine Laboratory, UMY Pharmaceutical Technology Laboratory, UGM Pharmaceutical Technology Laboratory, and UII Chemical Instrumentation Laboratory within 3 months.

2.2.Research variable and operasional definition

The independent variables of this study were the preparation of nanoemulsion extract and non-nanoemulsion extract from kapok banana peel and yellow watermelon peel. The dependent variables of this study were urea and creatinine levels in kidney stone models.

Nanoemulsion extract from kepok banana peel and yellow watermelon peel is a nanoemulsion preparation obtained from extracts made from powder or simplicia of kepok banana peel and yellow watermelon peel. Nanoemulsion is a colloidal particle system in the size range of 10-1000 nm which acts as a carrier for drug molecules[19][20].

Ureum and creatinine levels are indicators for assessing kidney function. Measurement of urea and creatinine can use blood urea and creatinine concentrations. If urea and creatinine are not excreted through the kidneys, urea levels in the blood will increase above normal levels because glomerular filtration must decrease to 50%. If the levels of urea and creatinine in the blood increase, this is an indication of kidney damage[21].

Urea and creatinine sampling methods. Rat blood samples were taken via the orbital vein with a capillary pipette, collected in a 1.5 cc EDTA tube. Samples were taken to LPPT UGM for urea and creatinine tests with Microlab 300 and production reagent kits. Ureum test using the Urease-GLDH method and creatinine test using the Jaffe method. Blood samples were taken on the 7th, 14th and 21st days [22]

2.3.Research Instrument

Measuring pipette (Pyrex), oven (Memmert), blender (Philips), knife, water bath, filter funnel, mortar, beaker (Pyrex), homogenizer, volumetric flask (Pyrex), test tube (Pyrex), rotary evaporator, scales analytical (KERNALJ), rat cage, and atomic absorption spectrophotometer (AAS).

Research Materials: Kepok banana peel, yellow watermelon peel, 21 male white Wistar strain rats, 70% ethanol, 0.1% HCl, rat food, ethylene glycol, ammonium chloride, aluminum foil, magnesium, distilled water, Tween 80, phosphate buffer , and glass bottles.

2.4.Research Procedure

2.4.1. Preparation of Kepok Banana Peel Extract

The kepok banana skin, which is greenish and slightly yellowish in color, is washed clean, cut into small pieces, and dried in a 50°C oven. 300 gr banana peel is mashed using a blender. Maceration using 1 liter of ethanol solvent and stirring. The container was closed and left for 3 days, stirring every 24 hours. The maceration results are filtered to obtain a filtrate. A thick extract is obtained after the filtrate is evaporated using a rotary evaporator[23].

2.4.2. Preparation of Yellow Watermelon Peel Extract

The yellow watermelon rind is washed clean, cut into small pieces, and dried in the oven at 50°C. A total of 300 grams of yellow watermelon rind is ground using a blender. Watermelon rind extract is made using the maceration method. Watermelon rind powder is soaked with 1 liter of 70% ethanol in a maceration vessel for 1 x 24 hours, while stirring and kept away from sunlight. The finished macerate is evaporated over a water bath, filtered using a filter funnel. It is concentrated using a rotary evaporator until a thick extract of watermelon rind is obtained[11].

2.4.3. Formulatiomn of Nanoemulsion of Kepok Banana Peel and Yellow Watermelon Peel Extract

The concentration of kepok banana peel and yellow watermelon peel extracts was made using the Homogenizer method at a speed of 1000 rpm [23]. Kepok banana peel and yellow watermelon peel extracts were prepared as an oil phase with a concentration of 20% (2 g of kepok banana peel extract and yellow watermelon peel extract in 10 ml of 96% ethanol). Phosphate buffer solution as the water phase is made

using NaOH dan or HCl until the pH of a sample of 100 ml is obtained by mixing kepok banana peel extract and yellow watermelon peel at a concentration of 20% and 30% with Tween 80 and then dissolving it in a buffer solution[24].

2.4.4. Preparation of experimental animals

Twenty-one male white rats were divided into seven groups. Each group consisted of three mice. Before the study, the test animals were acclimatized for 2 weeks, fed pellets, and drank sufficient water. Group I normal control. Group II-VII mice were treated as mice with kidney stones. The ethylene glycol used to form kidney stones is 0.75% (v/v). Ammonium chloride used to speed up the process of kidney stone formation is 2%. Group II, the positive control, was given kidney medication. Group III negative control. Group IV was given the extract. Group V was given nanoemulsion extract at a dose of 125 mg/kg bw. Group VI was given nanoemulsion extract at a dose of 250 mg/kg bw. Group VII was given nanoemulsion extract at a dose of 750 mg/kg bw.

2.4.5. Taking blood samples and measuring parameters

Rat blood samples were taken via the orbital vein with a capillary pipette, collected in a 1.5 cc EDTA tube. Samples were taken to LPPT UGM for urea and creatinine tests with Microlab 300 and production reagent kits. Ureum test using the Urease-GLDH method and creatinine test using the Jaffe method. Blood samples were taken on the 7th, 14th and 21st days[25].

2.5.Data analysis

The research began with measuring blood urea and creatinine levels on day 0 to determine the normal urea and creatinine levels of mice before treatment and after the mice were treated. After that, data analysis was carried out using the one way ANOVA statistical test at a confidence level of 95%. This test is used to determine whether there are differences between groups treatment and continued with a post hoc Least Significant Difference (LSD) test. Data were analyzed using the SPSS 23 program. Analysis of variance was determined by a P value > 0.05 = not significantly different, while a P value < 0.05 = significantly different. If there is a significant difference, continue with the Duncan test to determine the effect of administering kepok banana peel extract and yellow watermelon peel extract on urea and creatinine levels[26].

2.6.Research Ethics

This research has been declared ethically appropriate by the Ethics Committee of the Faculty of Medicine and Health Sciences, Muhammadiyah University of Yogyakarta with No. 035/EC-HC-KEPK FKIK UMY/VII/2023. This research was conducted by observing research ethical principles.

3 Results and Discussion

3.1. Research result

3.1.1. Extraction of Kepok Banana Peel and Yellow Watermelon Peel Extract

Yellow watermelon peel and kepok banana peel are extracted using the maceration method. In a maceration vessel, stir and protect from sunlight, watermelon rind powder is soaked with 1 liter of 70% ethanol for 24 hours (Figure 1). The macerate was evaporated over a water bath and filtered using a filter funnel. To obtain a thick extract of watermelon rind, a rotational evaporator was used. The macerate appears dark brown.

Figure 1. Macerate of of Kepok Banana Peel and Yellow Watermelon Peel

3.1.2. Nanoemulsion formulation of Kepok Banana Peel Extract and Yellow Watermelon Peel.

The homogenization process of kepok banana peel and yellow watermelon peel extract was made using an Ultra-Turrax homogenizer at a speed of 15,200 rpm.

Table 1 Extract Nanoemulsion Formulation

Material	Formula		Function
	F1	F2	
Oil Phase			
Kepok banana peel extract + watermelon ethanol 96% extract (ml)	20	30	As a dissolved substance
Tween 80 (g)	2	3	As surfactant
Water phase			
Buffer fosfat (ml)	78	67	As a solvent

The nanoparticle preparation was made through two phases, namely the oil phase using Tween 80 and then continued with the water phase with the help of phosphate buffer (Table 1).

3.1.3. Evaluation of the Characteristics of the Nanoemulsion of Kepok Banana Peel and Yellow Watermelon Peel Extract

Several evaluation tests were carried out on the two nanoemulsion preparation formulas to determine one of the best formulas, namely (Jusnita and Nasution, 2019):

3.1.3.1. Test particle size and PDI size

The purpose of particle size analysis and PDI test with the Particle Size Analyzer (PSA) is to determine whether the nanoemulsion meets the particle size standard of between 20 - 500 nm. PSA works by scattering laser light on sample particles, which can be detected quickly by a photon detector at a certain angle to measure the size of the preparation or sample particles.

While the purpose of polydispersity index analysis is to determine the uniformity of particle size distribution, the polydispersity index (PI) value of nanoemulsion samples indicates that the particle size is more stable or the structure is homogeneous. If the polydispersity index value is not more than 0.5, the globule distribution is not uniform[17][27].

Table 2 Size and PdI of Nanoemulsions

Formula	Nanoemulsion Size (nm)	PdI
FI 20%	246,9	0,618
F2 30%	248,3	0,921

The nanoemulsion particle size criteria, which ranged between 20 and 500 nm, were met by the particles of formulas 1 and 2. The PDI value of the nanoemulsion for both formulas showed a value greater than 0.5, which indicates that the nanoemulsion preparation of the formula extract was not uniform, because the index value good polydispersity does not exceed 0.5. The nanoemulsion particle size criteria, which ranged between 20 and 500 nm, were met by the particles of formulas 1 and 2. The PDI value of the nanoemulsion for both formulas showed a value greater than 0.5, which indicates that the nanoemulsion preparation of the formula extract was not uniform, because the index value good polydispersity does not exceed 0.5[23].

3.1.3.2. Viscosity Test

Test the viscosity of the nanoemulsion preparation using a rotational viscometer. A good nanoemulsion preparation has a viscosity value between 1 and 100 cP (Jusnita and Nasution, 2019). According to the viscosity measurement results, formula 1 (20 percent) has a viscosity value of 2.70 cP and formula 2 (20 percent) has a viscosity value of 3.36 cP. Thus, the viscosity range of nanoemulsion preparations has entered the criteria.

3.1.3.3. Test pH

Both formulas have a pH of 7 so they are safe to use as a basic ingredient for medicine because they correspond to the pH of the small intestine (6-7.24), which is the main organ that absorbs medicine[28].

3.1.3.4. Nanoemulsion type test

The nanoemulsion type test is carried out by dissolving the sample into the water phase (1:100) and the oil phase (1:100). If the sample dissolves completely in distilled water, then the nanoemulsion type is classified as oil in water (O/W), whereas if the sample dissolves completely in the oil phase, then the nanoemulsion type is classified as water in oil (O/W) (Fig 2).

Figure 2. Samples F1 and F2

3.1.4. Results of nephroprotective activity tests and preparation of experimental animals

Twenty-one male white mice with an average body weight of 150 grams and 2 months of age were used for testing. Mice were divided into seven groups consisting of three mice per group. Before research, test animals were acclimatized for 2 weeks.

Group I normal control. Group II-VII mice were treated as mice with kidney stones. Ethylene glycol 0.75% and ammonium chloride 2% are used to form kidney stones. Group II, the positive control, was given kidney medication. Group III negative control. Group IV was given the extract. Group V was given nanoemulsion extract at a dose of 125 mg/kg bw. Group VI was given nanoemulsion extract at a dose of 250 mg/kg bw. Group VII was given nanoemulsion extract at a dose of 750 mg/kg bw.

3.1.4.1. Ureum serum

Using a capillary pipette, blood samples from mice were taken via the orbital vein on days 0, 7, and 14. Samples were collected in 1.5 milliliter EDTA tubes. After that, it was put in a cool box and taken to the Integrated Research and Testing Laboratory at Gadjah Mada University. Creatinine test using the Jaffe method and testureum using the Urease-GLDH method. Serum urea level data was obtained from the average serum urea level in each research group which was measured using a UV spectrophotometer

Table 3 Average results of serum urea levels

Groups	Ureum level (mg/dl) (day)		
	0	7	14
Normal	68,8	5 4,9	55,9
Positive control	53,8	44	95,2

Negative control	53	33,3	44,3
KPE + WPE	52,5	34,9	74,3
KWMPENE 125 mg/kgBW dose	52,5	37,4	64,1
KWMPENE 250mg/kgbw dose	61,9	35	48,6
KWMPENE 750mg/kg bw dose	48,5	44,9	61

Notes: KPE = kepok peel extract; WPE=watermelon peel extract; KWMPENE = Kepok and watermelon peel extract Nanoemulsion

Table.3 shows an increase in urea levels in all groups above normal, namely 13.9-28.3 mg/dL[22].

3.1.4.2. Serum creatinine

Data on serum creatinine levels were obtained from the average serum creatinine levels in each research group which were measured using a visible spectrophotometer.

Table 4 Average results of serum creatinine levels

Groups	Creatinine level (mg/dl) of day		
	0	7	14
Normal	0,47	0,48	0,34
Positive control	0,53	0,53	0,47
Negative control	0,39	0,51	0,41
KPE + WPE	0,54	0,55	0,39
KWMPENE 125 mg/kgBW dose	0,57	0,55	0,49
KWMPENE 250mg/kgbw dose	0,55	0,51	0,44
KWMPENE 750mg/kg bw dose	0,55	0,58	0,46

Notes: KPE = kepok peel extract; WPE=watermelon peel extract; KWMPENE = Kepok and watermelon peel extract Nanoemulsion

Table 4 shows the calculation results, it was found that the intervention group experienced a decrease in creatinine levels on the 7th and 14th days. This shows that intervention in the nanoemulsion and non-nanoemulsion groups can reduce plasma creatinine levels. On the 14th day, the average for the nanoemulsion and non-nanoemulsion groups was greater than the normal group. However, the administration of the extract had the lowest average compared to the nanoemulsion extract group at doses of 125 mg/kg bw, 250 mg/kg bw, 750 mg/kg bw.

Based on the results of the Friedmant test, it was found that the effect on urea and creatinine levels was significant with a p value <0.01 ($p<0.05$). This shows that administration of kepok banana peel and yellow watermelon peel extract nanoemulsion had no effect on reducing plasma urem and creatinine levels.

3.2. Discussion

3.2.1. Serum urea

The results of the study showed a significant increase in serum urea levels in all treatment groups. This is caused by several factors, such as variations in the condition of the mice in each group, the health condition of the mice, errors during the blood serum collection process or the mice experiencing stress during the research period which can be caused by external factors such as environmental conditions and cages or internal factors. Kidney damage occurs due to alkalinization of urine which results in the formation of urolithiasis resulting in structural damage to the kidneys and decreased kidney function[6] [22][29].

3.2.2. Serum creatinine

The results showed that creatinine levels decreased in the nanoemulsion and non-nanoemulsion intervention groups. This shows the effect of administering kepok banana peel extract and yellow watermelon peel nanoemulsion on reducing serum creatinine levels. This is thought to be because the extracts of Kepok banana peel and yellow watermelon peel contain flavonoid compounds. Flavonoids can be used to treat kidney damage because they can protect the body from free radicals through antioxidant mechanisms. Antioxidants can prevent damage and death of kidney tubular cells, which can stop the body from excreting creatinine from the body[12][11][30].

4 Conclusion

The administration of kepok banana peel and yellow watermelon peel extract nanoemulsion did not significantly influence the reduction in urea levels. Administration of kepok banana peel and watermelon peel extract nanoemulsion decreased creatinine levels in the nanoemulsion and non-nanoemulsion intervention groups. Based on the research results that have been obtained, the researcher provides suggestions for the continuation of further research, namely as follows:

1. The next research was carried out in a standardized experimental animal laboratory
2. Other factors can be considered, such as the comparison of the weight of each experimental animal and the feeding of the experimental animals.

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