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Preliminary Phytochemical Studies, GC-MS Analysis, and Anti-Nephrototic Activity of Ethanol Extract of *Phoenix dactylifera* L

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ABSTRACT. Date palm seeds (*Phoenix dactylifera* L.) are known to contain phenolic compounds that have the potential as natural nephroprotective agents. Phenolics with solvents suitable for polarity and compounds identified by phytochemical screening, GC-MS analysis, and activity assays. This research went through 3 stages, screening with exploration and qualitative analysis of GCMS. The anti-nephrotoxic activity test used a pre-posttest controlled group design. In this study, rats have divided into six groups, namely Healthy control, negative control, and three treatment groups with ethanol extract of dates 150, 300, and 600 mg/kg. Mice were induced with 1% carrageenan twice a week. On macroscopic observation, date palm seeds are oval with indentations and light brown with a hard texture. Microscopic profile, endosperm, parenchyma, endocarp, stone cells, collenchyma, starch, oil cells, and carrier bundles. Physicochemical analysis, water content (8%), ash content (1.15%), fiber content (14.96%), and ethanol soluble extract content (10.3%). GC-MS analysis showed phenol,2,6-bis(1,1-dimethyl ethyl)-4-methyl, 1,2-benzene dicarboxylic, and dihydromethyl jasmonate. A dose extract ethanol of 600 mg/kg BW reduces urea and creatinine levels up to 8,3 mg/dl and 0.132 mg/dl. The conclusion of the phytochemical study obtained a pharmacognostic profile. The GCMS analysis obtained the compound. The activity of the ethanol extract of date palm seeds can reduce urea and creatinine levels so that it developed as an anti-nephrotoxic alternative from natural ingredients.

Keywords: Creatinine, ethanol extract, *Phoenix dactylifera* L, phytochemicals, ureum

INTRODUCTION

Conventional medicine has received serious attention from researchers based on therapeutic principles (Abdelaziz et al., 2016; Al-Farsi & Lee, 2011). Compounds of plants had bioactivities such as antioxidant, anti-inflammatory, and anticancer (Besbes et al., 2004; Chaira et al., 2007). Compounds used in medicine and prescribed from plants reach 25%. Inflammation is an immune response to the stimulations of inflammatory cells that are overexpression at the molecular level (Antonelli & Kushner, 2017; Delphin et al., 2014). Inflammation plays a role in nephrotoxicity. The initiation of nephrotoxic substances causes the activation of inflammatory mediators in the inflammatory area that cause morphological or functional changes in the kidneys cells and cause a decrease in renal filtration function (Akcay et al., 2009; Golshan et al., 2017), About 20% of nephrotoxic drugs are antimicrobials, chemotherapeutic agents, and nonsteroidal anti-inflammatory drugs (Faught et al., 2018; Kim & Moon, 2012; John & Shobana, 2012). In addition, other compounds that trigger nephrotoxic events are carrageenan (Weiner, 1991; Meqbaali & Saif, 2016). Preventive to minimize the incidence of nephrotoxicity by utilizing natural ingredients, namely date palm seeds (Metoui et al., 2018; Orabi & Shawky, 2014). Date seeds have high antioxidants because they contain phenolic compounds such as polyphenols, flavonoids, tocopherols, vitamin E, and ascorbic acid (Abdelaziz & Ali, 2014; Al-Qarawi et al., 2008).

The high amount of antioxidants in date seeds is associated with anti-nephrotoxic activity, which functions as a free radical scavenger (Al-Qarawi et al., 2008). Tocopherol is an antioxidant compound that protects against nephrotoxicity by inhibiting gentamicin-induced lipid peroxidation. In addition, Tocopherol compounds as anti-inflammatory drugs, prevent stroke, increase bone growth, and strengthen nerves (Al-Qarawi et al., 2008; Pandya et al., 2018). Examination of urea and creatinine is one of the parameters for determining kidney function (Patel et al., 2013; Sakr et al., 2021), and examination of urea and creatinine by 67 mg/dl in gentamicin-induced mice (Saputri et al., 2017). The ethanol

extract of date palm seeds as conventional medicine is antineprototic (Saryono et al., 2018). This study, date palm seeds observing of macroscopically, microscopically, and physicochemically. Furthermore, the bioactive compounds were observed on the GC-MS profile to confirm the phytochemical compounds present and evaluate the anti-nephrotoxic activity in carrageenan-induced rats by looking at the decrease in serum creatinine and urea levels.

EXPERIMENTAL SECTION

Materials

Date seeds were supplied from the local market. The seeds are cleaned, dried, powdered, and stored in brown bottles. Aquadest, microscope, bunzen burner. Chloralhydrate, sulfuric acid, All the chemicals used were of analytical grade, purchased from Merck Chemical Company (Merck, Germany), Gas chromatography-mass spectrometry Shimadzu type QP 2010, male Wistar rats, creatinine kits, and urea kits.

Methods

Collection and authentication of plants

Date seed ingredients collected and purchased from local markets in the Purwoketo area. The authenticity of the plant by determination at the Faculty of Biology, Universitas Jenderal Soedirman Purwokerto with letter number 1678/UN.23.02.8/TA.00.01/2019, which states that date palm seeds are named *Phoenix dactylifera* L., family Araceae.

Macroscopic analysis

Dates seeds and simplicia were observed organoleptically on shape, color, surface, character, texture.

Microscopic analysis

Prepared cross-sections and simplicia powder from date palm seeds. The sample a glass object and drops of water and covered with a glass deck and then observed under a microscope with a magnification (40x, 100x). Cells like endosperm, parenchyma, endocarp, collenchyma, mesocarp are observed. Observation of simplicia powder was carried out with the powder placed on a glass object and dripped with chloral hydrate and covered with a glass deck, then heated over a Bunzen burner and then observed under a microscope with a magnification (40x, 100x). Cells such as collenchyma, oil cells, and starch. All images were photographed and recorded for further analysis.

Examination of physicochemical parameters

Drying shrinkage examination

One gram of powder was placed into a porcelain crucible at 105 °C for 30 minutes. The powder was at a temperature of 105 °C for 1 hour and cooled in a desiccator until the weight.

$$\% \text{ Drying srinkhage} = \frac{\text{Wet weight} - \text{dry weight}}{\text{Wet weight}} \times 100\%$$

Ash content analysis

Five hundred milligrams simplisia up to porcelain crucifix. The powder was put into a furnace temperature of 600 °C for 6 hours, in the desiccator, and weighed. Then the ash of the dried material content was determined.

$$\% \text{ Ash content} = \frac{C - A}{B - A} \times 100\%$$

Intormation:
A= Crucible weight empty
B= Crucible weight + sample before incubation
C= Crucible weight + sample after incubation

Determination of ash content which is not soluble in acid

Two-gram of the powder and 25 mL of dilute sulfuric acid were boiling for 5 minutes. The acid-insoluble was collected, filtered through an ash-free filter paper that was not soluble in acid to the dried material.

Determination of water-soluble essence

Five-gram simplicia was put in a clogged pumpkin, added 100 mL of saturated chloroform water, shaken repeatedly for the first 6 hours, then settled down for 18 hours, and then filtered. Twenty mL was evaporated and then dried in a shallow cup that has been heated at 105 °C and tapped. The remainder was heating at 1050 until fixed weight. The water-soluble (%) was determined.

Determination of ethanol-soluble extract

Five grams of powder was put in the clogged pumpkin, added 100 mL of 95% ethanol, shaken repeatedly for the first 6 hours, and settled down for 18 hours. The mixture was quickly filtered to avoid ethanol evaporation. Twenty filtrates were drying in a flat-bottomed dish that heated 105 °C, tamed, and heated the remainder at 105 °C until the fixed weight. The % ethanol-soluble extract was determined.

Analysis GC-MS

Phytochemical compounds on GC-MS analysis. Gas chromatography-mass spectrometry (GC/MS) on the Shimadzu Agilent Technologies 7890A GC system and the Agilent Technologies 5975C MS system, combined and equipped with an EI and a DB-5 (60m×0.25 mm) fused silica column with a film thickness. 0.25μm is required. Helium is the carrier gas for this analysis. The helium gas flow rate at 2 mL/min, with a split mode proportion of 1:100 used for sample injection of 1μL, and the MS analysis ionization voltage was controlled by the EI procedure at 70 eV. Samples of injected at a temperature of 100 °C. Phytochemical constituents by associating the mass spectrum results with the GC-MS database.

Animals and experimental designs

Wistar rats weighing 150-250 grams and aged 2-3 months from the Pharmacology Laboratory, Faculty of Pharmacy, University of Muhammadiyah Purwokerto, Indonesia. The animal handling protocol for this study followed the guidelines for laboratory animal care and approving by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, Jenderal Soedirman University, Indonesia (certificate ethical approval number: No. 082/KEPK/IV/2019). In this study, the design with pre-post controlled group design, and The rats into five groups, namely healthy control, negative control, and three treatment groups. All experimental animals were induced with 1% carrageenan twice a week for 14 days except for healthy control. The treatment of ethanol extract of dates seed (EEDS) at doses of 150, 300, and 600 mg/kg BW. and treatment dose is two times a day orally. Subsequently, blood samples were centrifuged at 3000 rpm for 10 min to obtain serum, and it is used the Jaffe method kit for measuring creatinine levels and the Berthelot method kit for urea levels.

Data analysis

Pharmacognosy and physicochemical of simplicia were analyzed descriptively qualitatively. GC-MS analysis descriptive analysis based on existing libraries on GC-MS equipment, nephrotoxic activity test using graphed prism 8 include statistical.

RESULTS AND DISCUSSION

Pharmacognosy Analysis

Macroscopic and microscopic analysis

Macroscopic observations include observing the shape of the date palm seeds, the shape of the cross-section, and the longitudinal of date seeds, texture, size, and color.

The results showed that the simplicia of date palm seeds had an oval shape with grooves, hard texture, light brown on the outside and gray on the inside of the date seeds, length 2.6 – 2.8 cm with a width of 1-1.125 cm (**Figure 1A and 1B**). Meanwhile, simplicia date palm powder has a rough texture, dark brown or brown (**Figure 1C**). The seeds of the Deglet Noor variant have a seed length of 2.54 cm and a seed diameter of 0.8 cm (Salomon et al., 2017), and are light brown to dark brown in color and oval in shape with grooves (Al-Farsi & Lee, 2011; Hamada et al., 2002). Microscopic observations include cross-sectional observations of date palm seeds and powdered simplicial.

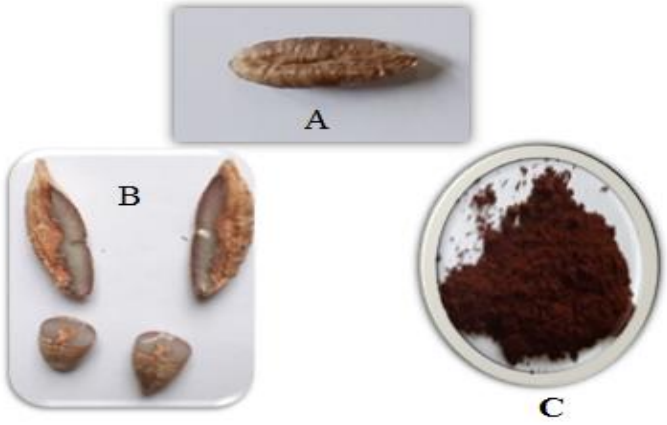

Figure 1. Results of macroscopic observations of date palm seeds simplicia. (A) Whole date seed simplicia, (B) Slice the date palm seed simplicia longitudinally and transversely, (C) Date palm seed powder simplicia.

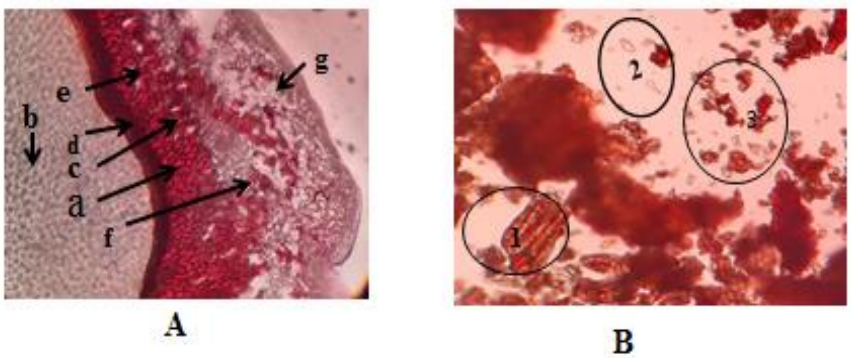

Figure 2. The microscopic observations of a cross-section of simplicia of date seeds. (**A**) and powder (**B**). (**Aa**) Endosperm, (**Ab**) Parenchyma, (**Ac**) Endocarpium, (**Ad**) Stone cells, (**Ae**) Collenchyma, (**Af**) Mesocarpium, (**Ag**). Epicarpium, **B1**. Collenchyma, **B2**. Starch grains, **B3**. Oil cell

In the cross-section, endosperm, parenchyma, endocarp, stone cells, myocardium, collenchyma, and epicardium tissue (**Figure 2A**). The powder showed oil cells, starch grains, and collenchyma (**Figure 2B**). The seed consists of outer seed coat tissue (epicardium), myocardium, endocarp, and seed core consists of embryo and endosperm.

Physico-chemical analysis

This analysis is pharmacognosy based on the physico-chemical properties of the Deglet Noor variant of the date powder simplicial (**Table 1**). Determination of water content to determine the durability of the sample in terms of storage. The low water content value indicates that the date palm powder can be stored for a long time without decay and is not susceptible to microbial growth of the biological activity of simplicia is well maintained (Jamil et al., 2010). Determination of ash content to identify the mineral content in the simplicia. Low ash content indicates total inorganic minerals. Inorganic minerals in high amounts can form complexes with metals in the bodies, because of enzymatic activity or other metabolic processes (Jamil et al., 2010). Determination of ethanol-soluble extract content how much extract or extract contained in the simplicia was soluble in ethanol solvent (Ramniwas & Singh, 2021). The high fiber content indicates that date palm seeds as a by-product as a good source of crude fiber for raw materials for fiber-based foods or supplements (Afifi et al., 2017).

GC-MS Analysis

Analysis compound and identification by a library of GC-MS, namely Willey 11. The spectrum of ethanol extract the compound 1,2-benzenedicarboxylic acid (**Figure 3** and **Table 2**), and IUPAC name called phthalic acid, is a class of terpenoid compounds and the type of aromatic dicarboxylic acid to have antioxidant activity.

The chemical formula $C_{16}H_{22}O_4$ has an area of 22.88 percent and appears at a retention time of 10,336. 1,2-benzenedicarboxylic acid compounds in brown algae extract potential as antioxidants (Thouri et al., 2017). 1,2-Benzenedicarboxylic acid compounds in *Polygonum chinense* L. have the potential as antioxidants, antimicrobials, and anti-inflammatory agents (Nagore et al., 2010). This dihydro methyl jasmonate compound (**Figure 4 and Table 2**) with the chemical formula $C_{13}H_{22}O_3$ has an area of 32.11% with a similarity of 98%, which has antioxidant activity. Weller & Wang, (2006) reported the presence of dihydro methyl jasmonate in Rubus sp, which increased its antioxidant activity and flavonoid content. Another study using LC-MS showed that the extract ethanol contains compounds 13-hydroxyabscisic acid, 1-ethylene-4-methylidene-2-oxo-7-(propane-2-yl)-octahydro1H-indent-5-yl-3-methylpent-2-enoic, acid-12-hydroxy-(8,10,14)-eicosatrienoate and 1-hydroxy-3-(pentadecanoyloxy) propane-2-yl-(5,8,11,14)-icose-5,8,11,14-tetraenoate (Siregar et al., 2018).

Table 1. Results of physico-chemical analysis of simplicia dates seed powder

Parameters	Consentration (%)	Standard	Reference
Water content	8	≤ 10	Depatemen
Ethanol soluble extract content	10.3	>10	Kesehatan RI, 1995
Ash content	1.5	≤ 10	
Acid soluble ash	0.2	≤1	
Ethanol soluble ash	0.4	≤1	Hamada, 2002
Fiber content	14.9	6.4-11.5	

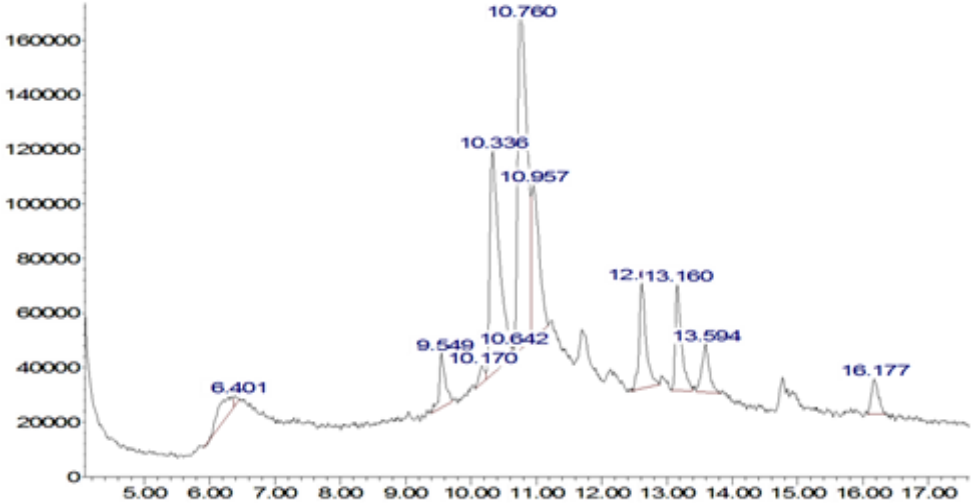

Figure 3. Profile GC-MS ethanol extract of date seeds

Table 2. Identified compounds of ethanol extract of date seeds

peak	RT	Compounds	MW	% Area	Similarity
3	9.549	Phenol, 2,6-bis(1,1- dimethylethyl)-4-methyl	220	2.28	91
4	10.336	1,2-Benzenedcarboxylic acid	330	22.88	97
5	10.763	Dihydro methyl jasmonate	226	32.11	98
	10.968	Cyclopentanetic acid	226	16.68	96
	12.618	7-Acetyl-6-ethyl-1,1,4,4-tetramethyleter	258	5.72	92
9	13.157	Methyl-3-(3,5-ditertbutyl-4- hydrxyphenyl) propionate	292	5.56	93

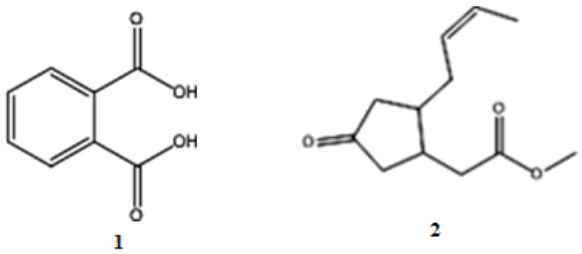

Figure 4. Structure 1,2-benzenedicarboxyl acid (**1**) and dihydro methyl jasmonate (**2**)

Anti-Nephrotoxic Activity Test

Urea and creatinine levels after carrageenan induction in the control and negative treatment groups were higher before the ethanol extract treatment in the healthy control group (**Figures 5 and Figure 6**). Urea and creatinine levels in healthy controls were 50 mg/dL and 0.472 mg/dL. One-way ANOVA analysis showed a significant difference ($P<0.5$) between the healthy group and the other four groups by carrageenan-induced. There was urea and creatinine in healthy controls and negative controls after 14 days. Mice in the healthy group did not receive any treatment and did not affect urea levels. The healthy control group experienced an increase in urea from 50 mg/dL to 53.4 mg/dL and creatinine by 0.472 mg/dL to 0.5 mg/dL, and Increased muscle mass and biological systems in rats (Guyton & Hall, 1997; Saka et al., 2012). However, this increase was not much higher than in the treatment group. In another study, the Healthy control compared to the treatment group gave almost the same anti-inflammatory effect (Saryono et al., 2018) In the healthy group, the increase was appropriate, proving that carrageenan can significantly increase urea and creatinine levels. Decreased levels are given ethanol extract of date palm seeds for 14 days (**Figure 5 and Figure 6**). The results showed a decrease in urea levels from 71.7 ± 2.03 mg/dL to 60.9 ± 2.21 mg/dL and creatinine levels from 0.68 ± 0.189 mg/dL to 0.548 ± 0.174 mg/dL. significant based on the Paired T-test. The ethanol extract of date seed had anti-nephrotoxic activity by reducing urea and creatinine levels.

The urea and creatinine in healthy control have levels reaching [-7.9 mg/dl] and [-0.148 mg/dl] because the posttest results are higher than the pretest results. This indicates an increase in posttest urea levels in the carrageenan-only induced group. Meanwhile, in the group giving Ethanol extract of date seed (EEDS), the data on the difference of urea showed positive results. In the EEDS group, there was a decrease in posttest urea levels due to the administration of ethanol extract of date palm seeds (**Figure 5**). The results of the one-way ANOVA test between the negative control group and the EEDS 150, EEDS 300, and EEDS 600, respectively, the levels of creatinine difference were 0.058 mg/dl; 0.05 mg/dL, and 0.132 mg/dL, that the hight decrease in creatinine levels was in the EEDS 600 group with an EEDS dose of 600 mg/kg BW (**Figure 6**). Supported by one-way ANOVA test $p>0.05$ for groups 3 (dose of 150 mg/kg BW) and 4 (dose of 300 mg/kg BW). The doses 150 and 300 mg/kg BW have the same ability. The lower compared at a dose of 600 mg/kg BW showed a p-value <0.05 or a significantly different ability to reduce urea levels. Doses a 600 mg/kg BW EEDS has the best ability to reduce creatinine up to 0.132mg/dL in the blood serum of rats induced by carrageenan.

This study of the administration of ethanol extract together with the induction of nephrotoxic substances caused a significant decrease in urea and creatinine in levels, namely 34.77 mg/dL and 0.6 mg/dL, which proves that date palm seeds have anti-nephrotoxic activity (Al-Qarawi et al., 2008). EEDS dose of 600 mg/kg BW can reduce the best urea and creatinine levels in carrageenan-induced rats so that it has anti-nephrotoxic activity. In this study, urea and creatinine levels of the H-0 and the effect of rat feeding, the feed given to the rats was all-feed 4 mm and contained 14-16% protein, 4-6% fat, 4-6% fiber, and 9-10% water content, and protein of mice 12% (Nisha et al., 2017). A feed of rats exceeds the normal urea and creatinine levels H-0.

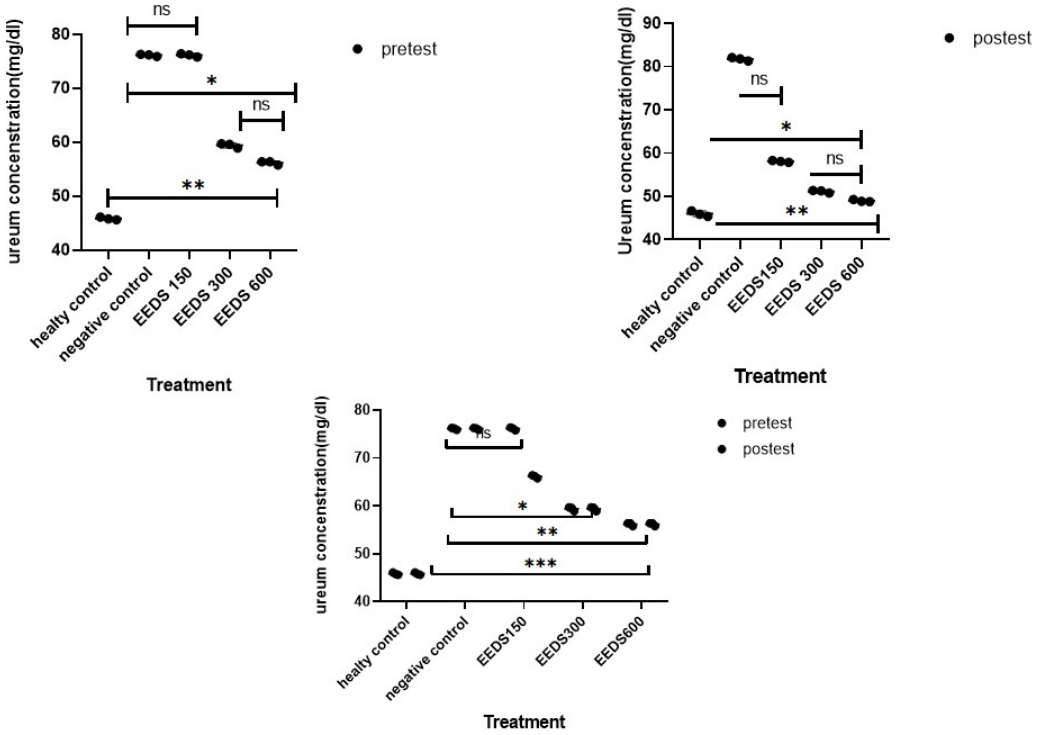

Figure 5. Profile of urea levels pretest and posttest in blood serum of rats induced by carrageenan. Healthy control was significantly different from negative control and treatment. (p, 0.05), the negative control was significantly different from the extract treatment (P, 0.05), the treatments were not significantly different. EEDS 150 (Ethanol extract of date seed dose 150 mg/BW), EEDS 300 (Ethanol extract of date seed dose 300 mg/BW), EEDS 600 (Ethanol extract of date seed dose 600 mg/BW).

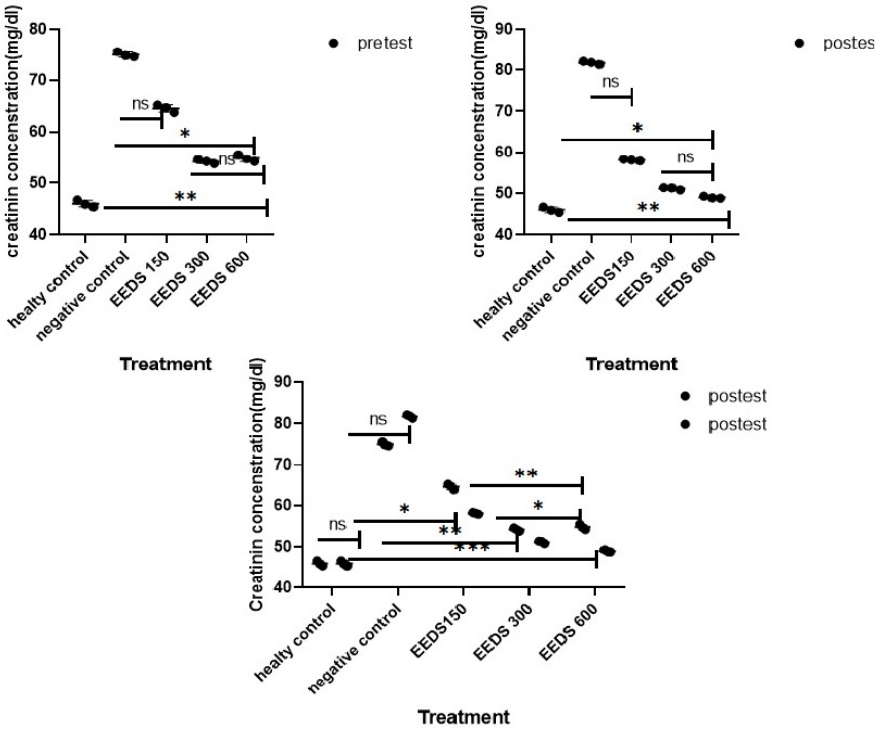

Figure 6. Profile of creatinin levels pretest and posttest in blood serum of rats induced by carrageenan. Healthy control was significantly different from negative control and treatment. (p, 0.05), the negative control was significantly different from the extract treatment (P, 0.05), the treatments were not significantly different. EEDS 150 (ethanol extracts of date seed dose 150 mg/BW), EEDS 300 (ethanol extracts of date seed dose 300 mg/BW), EEDS 600 (ethanol extracts of date seed dose 600 mg/BW).

The Date seeds in the palm are flavonoids, saponins, and tannins (Suwito et al., 2017). A Quercetin of the activated renoprotection with a free radical mechanism and lipid peroxidation (Ramniwas & Singh, 2021). It also contains palmitic acids, N-isobutyldeca-trans-2-trans-4-dienamide (Sudjarwo et al., 2017; Faught et al., 2018). The piperine was proven to improve kidney histopathology and reduce BUN creatinine in rats (Sudjarwo et al., 2017). The ureum in the blood serum by several factors. A pathological factor of acute and chronic kidney damage and a treatment factor of animal feeding. According, an increase in blood urea levels of kidney damage only is accompanied by a urine examination (urinalysis) by clinical signs that support the diagnosis of kidney failure. Creatinine levels in blood serum are unaffected by food, age, sex, activity, and diet (Faught et al., 2018; Sumarny et al., 2006). It is the excretion in the urine through renal or glomerular filtration, kidney damage, and creatinine will accumulate in the blood can be used to diagnose kidney disorders by measuring the glomerular filtration rate. Gentamycin and captopril accumulated in the renal cortex, and kidney cells. Cause oxidative stress, lysosomes, damaged area, urea, and creatinine. Blood Urea Nitrogen (BUN) and creatinine are waste products from normal metabolism in the urine, and low levels in the blood, and the kidney function or BUN, and creatinine excreted by the kidneys were accumulating in compounds in the blood, a parameter for kidney function (Faught et al., 2018).

One cause of urea and creatinine is the presence of excessive free radicals and an impact on kidney cell damage. In this study, nephrotoxic mechanism gentamicin, captopril, and antioxidants from African leaves (*Vernonia amygdalina*). Antioxidants are protection against nephrotoxicity due to free radicals and reactive oxygen species (ROS) that induce oxidative stress in the kidney. Free radical and ROS will cause cell damage, fibronectin protein in the lumen of the kidney tubules, and cause blockage of the lumen that urea and creatinine levels (Muhyi et al., 2014). Research on the administration of mangosteen peel extract reduces the increase in serum urea and creatinine levels in male rats induced by isoniazid.

CONCLUSIONS

On macroscopic observation, date palm seeds have an oval shape with grooves, light brown, L: 2.6-2.8 cm, L: 1-1.125 cm, and a hard texture. Endosperm, parenchyma, endocarp, stone cells, mesocarp, collenchyma, epicarp, and seed coat tissue under a microscope. The date seed powder is brown and coarse. The microscopically showed visible starch granules, oil cells, and carrier bundles. Date palm seeds have 8% moisture content, 1.15% ash content, 14.96% fiber content, and 10.3% ethanol soluble extract content. Date seed ethanol extract has the best anti-nephrotoxic activity with the best reduction in blood serum urea and creatinine levels of rats induced by carrageenan at a dose of 600 mg/kg BW.

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