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Research Article
Effects of Nanoherbal Haramonting (*Rhodomyrtus tomentosa*) and Extra Virgin Olive Oil on Histology of Liver and Kidney of Preeclamptic Rats

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Abstract

Background and Objective: Preeclampsia (PE) is a life threatening disease prevailing in pregnant women in Indonesia. Impaired kidneys and livers function in PE has a high risk during pregnancy. This study aimed to determine the role of nanoherbal haramonting and Extra Virgin Olive Oil (EVOO) on kidney and liver safety in preeclampsia rats. Nanoherbal *Rhodomyrtus tomentosa* is a medicinal plant with antioxidant activity such as EVOO which acts as inhibitor of oxidation, reduces lipid peroxidation and increases the speed of epithelialization. **Materials and Methods:** This study used pregnant rats (*Ratus norvegicus*) consisting of 5 treatments. Animal models of preeclampsia were made by injection of NaCl 6% 3 mL/day/kg b.wt., subcutaneously in pregnancy of 6-12 days and then given herbs in pregnancy at 13-19 days. The study group consisted of, C-: Normal pregnant rats, C+: PE rats, T1: PE rats were given EVOO, T2: PE rats were given nanoherbal haramonting and T3: PE rats were given EVOO and nanoherbal haramonting. Tissue histology was made by the paraffin method and staining Hematoxylin Eosin which was dissected on the 20th day of pregnancy. **Results:** There was no significant difference in weight of the liver and kidneys after administration of EVOO and nanoherbal haramonting. The administration of this herb significantly decreased the narrowing of the kidney tubules and the glomerular diameters. Both of these herbs also repaired preeclamptic liver damage ($p < 0.05$) in normal hepatocyte cells, parenchymal degeneration and necrosis. **Conclusion:** The EVOO and nanoherbal haramonting repaired preeclamptic liver and kidney damage.

Key words: Preeclampsia, *Rhodomyrtus tomentosa*, Extra Virgin Olive Oil (EVOO), liver, kidneys, nanoherbal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Preeclampsia (PE) is a dangerous and potentially life-threatening disease for pregnant women and fetuses in Indonesia. Monitoring renal function appears to be relevant for PE, especially since albuminuria must be evaluated postpartum and classified as being at high risk for impaired renal function[1]. Liver and kidney disease in PE has a high risk of pregnancy disorders, although until now there have been no reports of maternal deaths but of premature baby births or neonatal deaths[1-3]. According to Situmorang *et al.*[2], the combination of nanoherbal andaliman and Extra Virgin Olive Oil (EVOO) can affect kidney safety and the percentage of tubular constriction. Liver dysfunction in preeclampsia is a specific predictor that can affect the mother and fetus. The potential of liver biomarkers in predicting the incidence and outcome of preeclampsia is beneficial in reducing and preventing this disease, because liver dysfunction in preeclampsia patients in severe conditions affects the fetus[4]. Prevention of liver and kidney damage has been tried with the use of baicalin as a therapeutic candidate for the PE mouse model[5]. However, traditional Indonesian people still believe in herbal medicines because they have fewer side effects. In a retrospective analysis, PE patients can also have problems with serum creatinine and the results showed that it determines renal function and comorbidity[6]. Increased awareness of kidney health in pregnancy such as; Chronic Kidney Disease (CKD) is very important. It can cause complications up to 3% of pregnancy and PE is associated with an increased risk for the development of kidney disease[7].

Rhodomyrtus tomentosa (Haramonting) is a medicinal plant that have biological effects including immunomodulator activity in humans and mammals such as the immunomodulation effect *in vitro*[8]. Haramonting leaves have great antioxidant activity as oxidation inhibitors. Haramonting leaves (*Rhodomyrtus tomentosa*) contain flavonoids, saponins, tannins and triterpenoids. The content of flavonoids are a powerful antioxidant that could reduce lipid peroxidation, increase the speed of epithelialization and anti-microbial[9,10]. The effect of administration of haramonting leaves extract also affects the alveolar membrane, alveolar lumen and the relationship between alveoli[11]. The administration of nanoherbal haramonting significantly ($p<0.05$) affects the kidneys but if given excessive doses causes an increase in proximal tubular constriction in Mice[12]. Alcohol extract (70% water) from *Rhodomyrtus tomentosa* has hepatoprotective activity and reduce oxidative damage caused by antitubercular drugs[13]. *In vitro* antioxidant activity of haramonting extract inhibits lipid peroxidation, antioxidant power of reducing iron and chelating activity of metals. In addition, this plant extract also showed a reduction effect in free radicals in Albino mice[14]. However, administration of nanoherbal haramonting to the liver and kidneys of patients with preeclampsia has never been done.

In the context of this problem, a histological analysis of the kidneys and liver was conducted with special attention to preeclampsia and to tried to understand how these two organs improved pregnancy. Furthermore, analysis of the type of degree of liver damage and narrowing of the tubules was also explored in preeclampsia mice. The aim of the observation was to know that preeclampsia could damage and disrupt the liver and kidney metabolism system and be dangerous in pregnancy and to examine the function of organs and liver during pregnancy.

MATERIALS AND METHODS

The study was conducted at the Biology Laboratory of the University of Sumatera Utara, Pathology and Anatomy Laboratory of the University of Sumatera Utara and the Indonesian Institute of Education and Research, Jakarta (March-October, 2019).The experimental animals used were 50 pregnant rats (*Rattus norvegius*) from the Animal Cage of the University of Sumatera Utara.

Animal handling: This study consisted of 5 treatments using pregnant rats. Rats (PE) were injected 3 mL/day/kg b.wt., NaCl 6% subcutaneously at 6-12 days of pregnancy and then given herbs orally at 13-19 days of pregnancy. The group consisted of negative control (C^-): Pregnant rats without treatment, Positive control (C^+): PE rats, T1: Rats were given EVOO 1 mL/kg/day b.wt., T2: Rats were given nanoherbal haramonting 100 mg kg^{-1} b.wt., T3: Rats were given EVOO 0.5 mL/kg/day b.wt. and nanoherbal haramonting 50 mg kg^{-1} b.wt. Researchers have received permission from the Ethical Clearance Animal Management Faculty of Mathematics and Natural Sciences, University of North Sumatra (No. 281/KEPH-FMIPA/2019).

Nanoherbal haramonting: *Rhodomyrtus tomentosa* (Haramonting) leaves were obtained from a resident plantation in Rantauprapat, North Sumatra, Indonesia. Making of nanoherbal haramonting by High Energy Milling (HEM) tool at LIPI Jakarta[15]. The EVOO was from a supermarket in Medan.

Liver and kidney tissue: Making of tissue carried out in the Anatomy Pathology Laboratory of Adam Malik Hospital, Medan, Indonesia. The liver and kidneys of pregnant rats were made using the paraffin method. The stages of hematoxylin eosin staining started from the process of deparaffinization using xylol I, II and III solutions, respectively for 3-5 min then proceed with the rehydration process using multilevel alcohol starting with absolute alcohol, 90-30% each for 3-5 min. The tissue was stained with hematoxylin for 30-45 sec then rinsed with distilled water for a few moments. Furthermore, the tissue was stained again with eosin for 30-45 sec and then followed by a process of dehydration using multilevel alcohol starting with a concentration of 30-90% and absolute alcohol 5 times each. The tissue was clarified with xylol solution for 3 min. The next stage was the mounting process carried out by tissue closure, then observed using a stereo microscope and OptiLab tool with 40x magnification.

Data analysis: The study data using SPSS software version 23 (ANOVA test) at a level of 5% and proceed the *post hoc* Duncan test. The data haven't distributed normally and using a non-parametric test (Kruskal Wallis test and then followed by the Mann-Whitney test).

RESULTS

Weight of liver and kidneys after giving nanoherbal haramonting and EVOO: There was no significant difference ($p>0.05$) in liver and kidney weight in pregnant rats in each treatment (Table 1). However, the highest liver and kidney weight found in the negative control group (pregnant rats without PE). The highest liver weight found in PE rats (negative group) and the lowest kidney weight was in T2 (PE rats by administration of nanoherbal haramonting). Haramonting affected the weight of the kidneys and liver although not proven significantly.

Description of kidneys tubules after giving nanoherbal haramonting and EVOO: There was a significant difference ($p<0.05$) to the narrowing of the tubules in the kidneys (Fig. 1). The tubules narrowed were highest in the positive control group (PE rats). The tubules narrowed were lowest in the negative control (Normal pregnancy). The highest narrowed tubules between treatments after given by the drugs were found in T3 rats (PE rats by administration of nanoherbal haramonting and EVOO) than T2 and T1 group. The combination of haramonting and EVOO influenced the narrowing of the kidney tubules significantly. The kidney

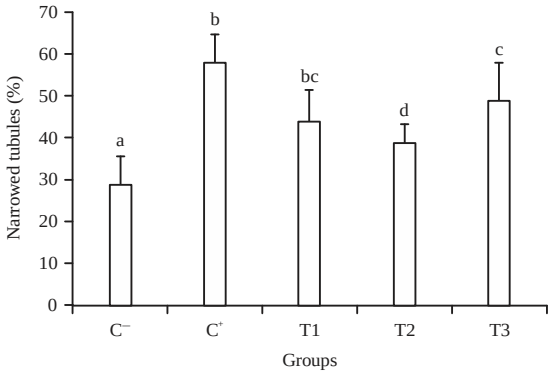

Fig. 1: Narrowed tubules

C⁻: Pregnant rats without treatment, C⁺: PE rats, T1: PE rats after giving EVOO, T2: PE rats after giving nanoherbal haramonting, T3: PE rats after giving EVOO and nanoherbal haramonting, different letter means significant difference at 0.05 probability

Table 1: Kidneys and liver weight after giving EVOO and nanoherbal haramonting

Groups	Liver weight (g)	Kidneys weight (g)
C⁻	7.678±0.372[a]	0.534±0.092[a]
C⁺	7.054±0.400[a]	0.526±0.071[a]
T1	7.384±0.393[a]	0.506±0.015[a]
T2	7.166±0.354[a]	0.466±0.032[a]
T3	7.572±0.494[a]	0.468±0.017[a]

Values are expressed as mean and standard error of mean, p-values were determined by ANOVA test, [a]Non-significant difference

tubules in the negative control group (C⁻) and positive control (C⁺) was different (Fig. 2a-e). Renal tubules of PE rats changed in the shape. Narrowing of the tubules also occurred beside the cells being necrosis or empty. The T1 group looked necrosis and tubules were narrowed but they were not as severe as the PE group (C⁺). The T2 also showed necrosis but it standed out was narrowed of the tubules. Proximal tubular epithelial cells were sensitive to anoxia. They destroyed easily and excreted through the kidneys. Tubular constriction was seen in the combination of the two plants. Based on the treatment group, administration EVOO in preeclampsia rats was better than the haramonting group and its combination.

Description of kidneys glomerular after giving nanoherbal haramonting and EVOO: There was a significant difference ($p<0.05$) in glomerular diameter in each treatment (Table 2). The highest glomerular diameter was found in the T3 group (PE rats were given nanoherbal haramonting and EVOO). The lowest glomerular diameter found in PE rats (positive group). Substances, compounds or drugs that in the body would be filtered by the kidney glomerulus. The activity could affect the diameter of the glomerulus depending on the incoming substance and the dose given.

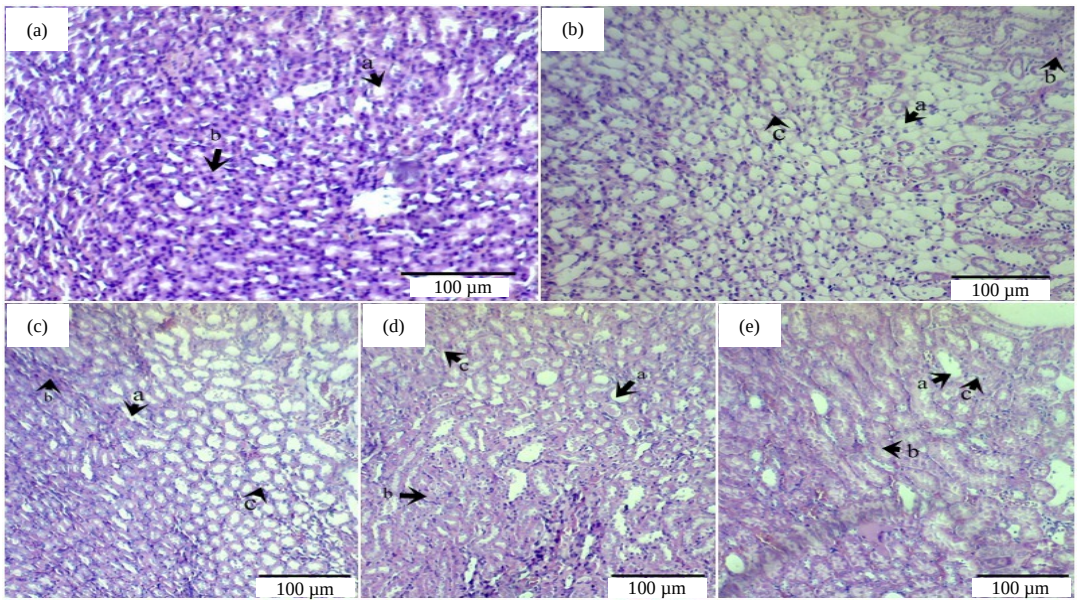

Fig. 2(a-e): Histology of narrowed tubules in, (a) C⁻, (b) C⁺, (c) T1, (d) T2 and (e) T3

C⁻: Pregnant rats without treatment, C⁺: PE rats, T1: PE rats after giving EVOO, T2: PE rats after giving nanoherbal haramonting, T3: PE rats after giving EVOO and nanoherbal haramonting, a: Normal tubules, b: Narrowed tubules, c: Necrosis (40×)

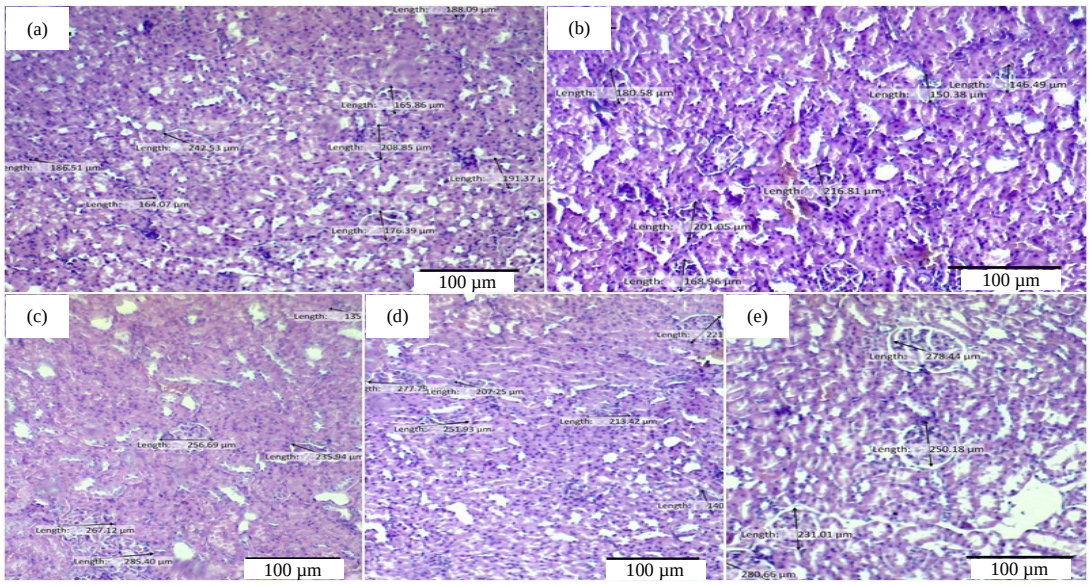

Fig. 3(a-e): Glomerulus diameters of, (a) C⁻, (b) C⁺, (c) T1, (d)T2 and (e) T3

C⁻: Pregnant rats without treatment, C⁺: PE rats, T1: PE rats after giving EVOO, T2: PE rats after giving nanoherbal haramonting, T3: PE rats after giving EVOO and nanoherbal haramonting, a: Normal tubules, b: Narrowed tubules, c: Necrosis (40×)

Table 2: Glomerulus diameters after giving EVOO and nanoherbal haramonting

Groups	Glomerulus diameter (µm)
C⁻	212.728±23.963^{ab}
C⁺	197.384±11.397^{a}
T1	214.401±17.733^{abc}
T2	233.919±7.568^{bc}
T3	238.437±20.978^{c}

Values are expressed as mean and standard error of mean, p-values were determined by ANOVA test, different letter means significance difference at 0.05 probability

The histology of glomerulus calculated using the Optip Lab tool. The glomerular shapes started irregularly in the PE group (C⁺) and changing in the shape and size of the diameter in the glomerular rats of PE affect the kidneys (Fig. 3a-e). Dosing was very important because it affected the activity of the glomerulus. Glomerulus played a role in the filtration system when it entred of substances or compounds in the body.

Description of liver histology after giving nanoherbal haramonting and EVOO: The administration of nanoherbal haramonting and EVOO affected the value of liver damage and liver histology. There were significant differences ($p<0.05$) in normal hepatocyte cell values, parenchymal degeneration and necrosis (Fig. 4). However, there was no significant difference in hydrophic degeneration ($p>0.05$). This study found that the administration of haramonting in preeclampsia livers significantly affected and thought to be developed into a future drug but further research and analysis needed to be done. The highest of normal hepatocyte cells was C^- groups and the lowest was C^+ (Fig. 5a-e). The highest parenchymal degeneration found in C^+ group and the lowest in T2 group (nanoherbal haramonting). The highest hydrophic degeneration found in T3 group (combination of EVOO and nanoherbal haramonting) and the lowest was found in C^+ group. The highest liver cell necrosis found in C^+ group (PE) and the lowest was in C^- (Control). The liver was an organ that plays a role in detoxification of poisons. Then substances or compounds entered the body would be neutralized by the liver. However, hepatocyte cells in the liver could be damaged due to the presence of foreign substances at inappropriate dosages. The health of organs and liver was very important in preeclampsia because they affect the metabolic system in the body.

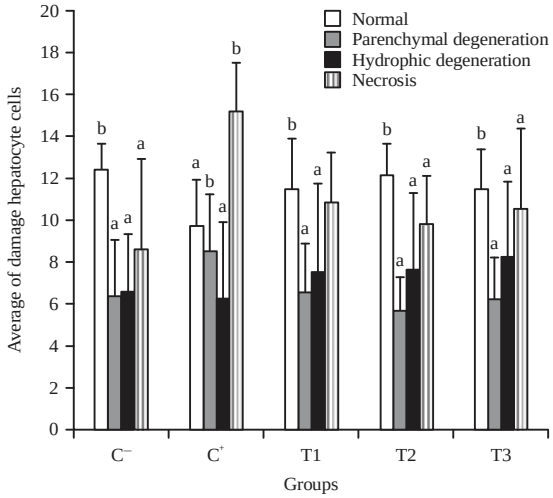

Fig. 4: Average of damage hepatocyte cells

C^-: Pregnant rats without treatment, C^+: PE rats, T1: PE rats after giving EVOO, T2: PE rats after giving nanoherbal haramonting, T3: PE rats after giving EVOO and nanoherbal haramonting, different letter means significant difference at 0.05 probability

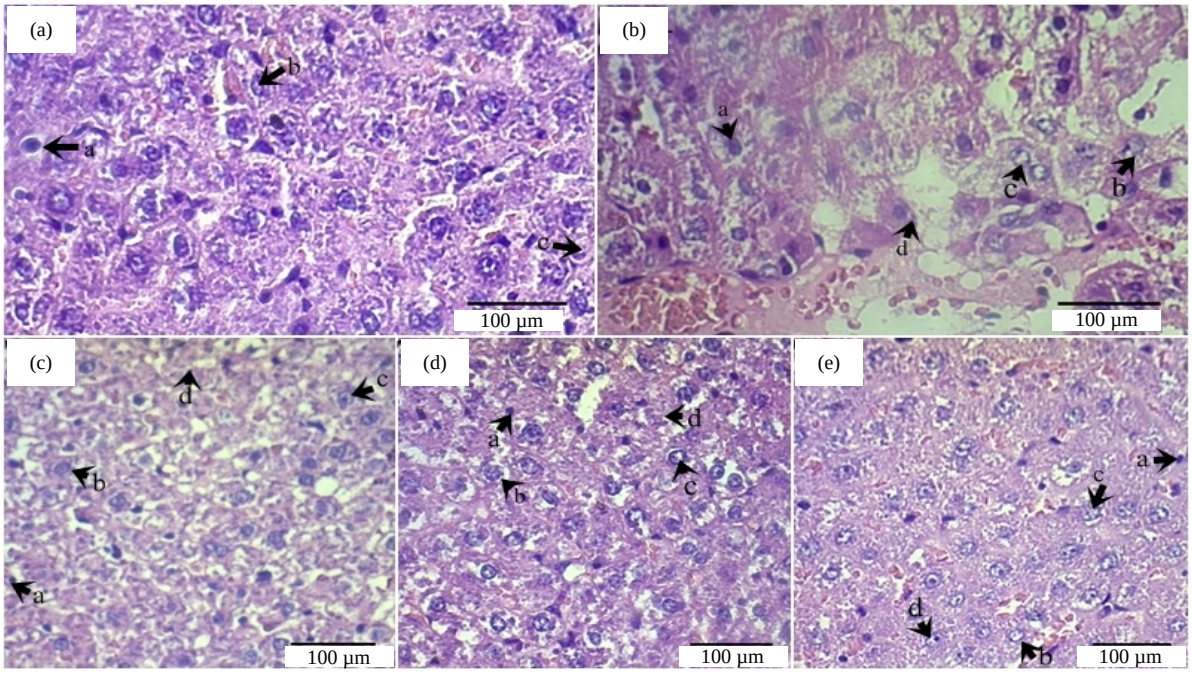

Fig. 5(a-e): Histology of liver cells in, (a) C^-, (b) C^+, (c) T1, (d) T2 and (e) T3

C^-: Pregnant rats without treatment, C^+: PE rats, T1: PE rats after giving EVOO, T2: PE rats after giving nanoherbal haramonting, T3: PE rats after giving EVOO and nanoherbal haramonting, a: Normal hepatocyte, b: Parenchymal degeneration, c: Hydrophic degeneration, d: Necrosis (40x)

DISCUSSION

This study found that nanoherbal haramonting, Extra Virgin Olive Oil (EVOO) and the combination of these two herbs can influence the liver and kidney histology of patients with preeclampsia. Study of rat liver after given haramonting extract showed hepatoprotective activity and reduced oxidative damage caused by antitubercular drugs[13]. However, the haramonting was changed to nano size. Nanotechnology in herbal medicine can be targeted at body organs such as the brain, lungs, kidneys and digestive tract because it has high effectiveness and safety[11,12].

Narrowing of the kidney tubules in preeclampsia also depended on the dose of the drug or substances entered the body. Excessive substances would be accumulated in the kidneys so that high blood flow to the kidneys, causing various drugs and chemicals in the systemic circulation[16]. Proximal tubular epithelial cells were sensitive to anoxia and destroyed easily. They were excreted through the kidneys (Fig. 2a-e), which could cause pale cortex, enlarged kidney, edema, pyramidal congestion and cytoplasm of vacuolated cells in the proximal tubules[17]. Haramonting extract could be act as a powerful antioxidant and they used as an antioxidant supplement in food products. Oxidative stress was believed to be one of the main causes of human disease that could occur in severe cellular dysfunction due to membrane lipid peroxidation, protein modification, nucleotide nicotinamide depletion, rise in intracellular free calcium ions (Ca^{2+}), cytoskeletal disorders and DNA damage[18,19]. To prevent this problem, haramonting acted as an alternative antioxidant therapy in the pharmacological industry to prevent free radical and biomolecular damage that occurs in many diseases of human organs. Necrosis could occur due the damage of tubular cells in the kidney and leakage of residual substances that were intratubular into the peritubular circulation. Kidney was vulnerable to damage because the kidney receives a large blood flow which is 25% of the resting cardiac output. This causes blood flow from the systemic circulation containing toxic substances to pass through the kidneys to be filtered and the mechanism of renal excretion disrupts by drugs or toxins[20]. Kidney cell damage could be caused by toxicity direct to cells (ischemia). Hemoglobin and ischemia cause the entry of extracellular calcium across the plasma membrane, followed by the release of calcium from intracellular deposits. The damage of kidney cells occurs due to the presence of toxic substances. They were estimated to be inhibited by the antioxidant activity possessed and by phenol and flavonoid compounds in haramonting leaves.

Plants have high antioxidants could be used to delay or prevent oxidation of cellular substrates. Antioxidants could be acted as hydroxyanisole and hydroxytoluene butylated to be hepatotoxic and carcinogenic[20]. The liver was the target organ of toxic substances through the digestive system then after it was absorbed. It was carried by portal veins to the liver. The highest parenchymal degeneration occurred in PE rats liver and lowest in the control group (normal pregnancy). Parenchymal degeneration (Fig. 5b) were the mildest level of the degeneration category and they were found in granules in the cytoplasm which causes the cytoplasm to become turbid and swollen in cells[21]. Administration of ethanol extract haramonting (*Rhodomyrtus tomentosa*) significantly ($p<0.05$) at a dose of 100 mg kg^{-1} b.wt., could affect to hepatocyte cells exposed to cigarette smoke[22]. Haramonting leaves extract (*Rhodomyrtus tomentosa*) could maintain normal values of leukocyte counts and affect the alveolar embryo, alveolar lumen and its relationship between alveoli[11].

The implication of the research is the examination of the liver and kidneys in pregnancy required for preeclampsia or healthy pregnancy. The herbs in this study can be applied as a material consideration in the health of pregnancy. Further research such as immunohistochemical analysis and mRNA in the liver and kidneys after administration of this herb is highly recommended, because the limit of this study only to the histology and the degree of damage.

CONCLUSION

The administration of nanoherbal haramonting and EVOO affected the histology of liver and kidney in preeclampsia. Nanoherbal haramonting and EVOO could improve to changes in renal tubular shape and glomerular diameter ($p<0.05$) while it reduced damage of cell hepatocytes such as parenchymatosa degeneration and necrosis, but they were not significant in hydrophic degeneration ($p<0.05$) in the liver.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of nanoherbal haramonting and EVOO combination that can be beneficial for preeclampsia rats. This study will help researchers to uncover that this herb in nano size reduces side effects as compared to extract and can provide effects that may be beneficial in liver and kidney preeclampsia. Liver and kidney function are also biomarkers of preeclampsia and the metabolic system of the liver and kidneys can be damaged by preeclampsia. Thus, a new theory on these herbal combinations and possibly other combinations, may be arrived at.

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