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Research Article
Role of Heat Shock Protein 70 (HSP-70) after Giving Nanoherbal Haramonting (*Rhodomyrtus tomentosa*) in Preeclamptic Rats

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Abstract

Background and Objective: Haramonting (*Rhodomyrtus tomentosa*) is an alternative herb to improve health because it has many biological activities and antioxidant. HSP-70 levels as biomarkers of preeclampsia affected the anti-apoptosis of damaged cells in the placenta. This study aimed to evaluate the role of HSP-70 expressions by investigating whether effect haramonting leaves in PE rats. **Materials and Methods:** The study design was control (C): pregnant rats without treatment, PE: Preeclamptic rats, PE+E: PE rats were given 1 mL EVOO kg^{-1} b.wt./day orally (pregnancy 13-19), PE+H: PE rats were given nano herbal haramonting 100 mg kg^{-1} b.wt. (pregnancy 13-19 days). PE+E+H: PE rats were given EVOO 0.5 mL kg^{-1} b.wt. and nano herbal haramonting 50 mg kg^{-1} b.wt. (pregnancy 13-19 day). Surgery was performed by taking blood from the heart for the SGOT/SGPT parameters, creatinine and HSP70. **Results:** A significant difference was observed in all groups with the value $p<0.0001$ and HSP-70 Expressions affect in preeclamptic rats after given this herbal. The value of SGOT, SGPT and creatinine can affect preeclamptic rats and can be as a biomarker of preeclampsia. A significant difference also in fetus weight ($p<0.01$) but an insignificant difference in placental weight ($p>0.05$). **Conclusion:** These findings indicate that Nano herbal haramonting and EVOO possess antioxidative effects and a promising drug for the future in the treatment of preeclampsia.

Key words: Haramonting, HSP-70, nanoherbal, preeclampsia, *Rhodomyrtus tomentosa*, SGOT and SGPT, creatinine

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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

Heat Shock Protein 70 (HSP-70) is essential for protein folding, translocation across the cellular compartment, assembling and maintaining multi-protein complexes[1]. HSP70 levels are increased in preeclampsia[2,3]. An increased mitochondria activity along with a decreased antioxidant during preeclampsia. Increased levels of HSP70 in preeclamptic patients or during pregnancy can cause hepatocellular injury, oxidative stress and systemic inflammation. HSP70 is from ischemic and placenta PE, which was oxidatively suppressed[5]. The HSP70 levels were significantly higher of the hemolysis syndrome, elevated liver enzymes and low platelet count (HELLP syndrome) than preeclamptic[2,3]. HSP affected and repaired the anti-apoptosis of damaged cells. Preeclampsia can cause apoptosis and disorders of the fetus. The increased malondialdehyde (MDA) as a sign of increased free radicals.

Rhodomyrtus tomentosa (Aiton) Hassk is a plant in the Myrtaceae family from the south and southeast Asia. It was used as a traditional medicine for diarrhea diseases, dysentery, gynecopathy, abdominal pain and wound healing. Its biological activities such as antioxidants, antibacterial, anti-inflammatory and anticancer[6]. Haramonting is an alternative way to control disease and improve health. It has biological activities such as antibacterial activity, antistress and growth-promoting effects[7,8]. Extra Virgin Olive Oil (EVOO) can effective in increasing plasma antioxidant activity and reducing MDA levels[9]. EVOO can reduce oxidative stress can increase the production of HSP-70 in cells[4]. EVOO increased HSP-70 production in cells because it contains antioxidants and tocopherol (vitamin E)[9-13].

In this study, we focused on the role of HSP-70 expressions by investigating whether effect haramonting leaves in PE rats. In the setting of our experiments, we've given Extra Virgin Olive Oil (EVOO) to 1 group for the rats PE as pure antioxidants and the comparison with haramonting. The antioxidants of EVOO is important from health, biological and sensory points of view. It has been proven by many researchers. Lipophilic, phenols and hydrophilic (include a large variety of compounds) represent the main antioxidants of EVOO[14]. EVOO can protect against induced cell injury through oxidative stress, inflammatory and apoptosis in rats model[15,16]. The aim of this study to analyze the role of HSP-70 expressions and investigating whether the effect haramonting leaves in PE rats.

MATERIALS AND METHODS

Nanoherbal haramonting and EVOO: Haramonting leaves from residential residents in Rantauprapat Sumatera Utara, Indonesia. Nanoherbal haramonting was made using high energy milling (HEM-3D, Kokusan H-103n, Japan) to nano-size[17,18]. Pure EVOO was purchased from supermarkets in Medan (Bertolli, Italia; Sertifikat: IFS-BRC). EVOO dose calculation was based on the previous studies[9,21].

Animal handling: This study used 25 Wistar pregnant rats from the Animal House of Biology Laboratory, the University of Sumatera Utara (USU), Indonesia. This research project was conducted from June, 2019-May, 2020 in Department of Biology, Physiology Laboratorium, Universitas Sumatera Utara, Medan, Indonesia. This study consisted of 5 treatments. The preeclampsia rats model was by injecting NaCl 6% 3 mL/day/kg b.wt., subcutaneously at pregnancy 6-12 days[4,17,19-21]. The study design was Control (C): Pregnant rats without treatment, PE: Preeclamptic rats, PE+E: PE rats were given 1 mL EVOO kg^{-1} b.wt., day^{-1} orally (pregnancy 13-19), PE+H: PE rats were given nano herbal haramonting 100 mg kg^{-1} b.wt. (pregnancy 13-19 days). PE+E+H: PE rats were given EVOO 0.5 mL kg^{-1} b.wt. and nano herbal haramonting 50 mg kg^{-1} b.wt. (pregnancy 13-19 day)[17,19]. The Surgery took the placenta, fetus and blood from the heart for the SGOT/SGPT parameters, creatinine and HSP70. The organ scales were used for rat's placenta and fetus. Researchers received permission from the ethical clearance of animal handling at the Faculty of Mathematics and Natural Sciences, University of Sumatera Utara (No. 010/KEPH-FMIPA/2020).

Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT): The rat's blood was centrifuged 15 min with a speed of 5.000 rpm. The blood serum and 200 µL aqua were put in the test tube. About 2000 µL of SGOT 1 reagent was added and incubated for 5 min at 37°C. A total 500 µL of SGOT 2 reagent was added and measured in 365 nm wavelength with a spectrophotometer (merk: LAMBDA™ 365, manufactured by Perkin Elmer, OHIO, United States). The blood serum for SGPT measuring was put in 200 µL of aqua into the test tube, then added 2000 µL of SGPT 1 reagent and incubation for 5 min at 37°C. The 500 µL of SGPT 2 reagents were added and measured in wavelength 365 nm with a spectrophotometer[22].

Creatinine: Total 3 mL of rat's blood samples centrifuged to separate serum from plasma. The standard blank solution and creatinine were prepared in advance. About 50 µL of blood serum and 1000 µL reagent 1 (NaOH) were put to the cuvette, mixed them and incubated for 5 min. About 250 µL of Picric acid and the solution were added, incubated for 1 min and measured on a spectrophotometer with a wavelength of 546 nm. A 50 µL of serum and 1000 µL of reagent 1 (NaOH) were added to the cuvette, the mixture then incubated for 5 min. Total 250 µL of picric acid reagent was added and measured using a spectrophotometer with a wavelength of 546 nm.

HSP-70 expressions: The rat's blood samples were centrifuged at 3000 rotations per minute for 15 min. The HSP-70 was examined using the Well Reader-Elisa Reader (R-Biopharm, a low-speed DT5-6A centrifuge, Darmstadt, Germany). The 100 mL of buffer solution is put in Petri dishes, then closed and incubated for 2 hrs at room temperature. About 100 mL of HSP-70 antibody solution pipetted into each dishes then incubated and stirred for 1 h at room temperature. The results read with a spectrophotometer wavelength of 450 nm[23].

Statistical analysis: The research data were analyzed using one-way ANOVA in Sigmaplot software. Data were analyzed with averages and standard deviations. Asterisks indicate the level of statistical significance (*p<0.05, **p<0.01, ***p<0.001 ****p<0.0001 and ns is equal to p>0.05).

RESULTS

SGOT, SGPT and creatinine results: A significant difference (ANOVA results) with a value of F<0.0001 in the SGOT value was obtained (Fig. 1a). An insignificant difference was observed in the control and PE (F = 0.0831, p>0.05) but a significance was observed in the PE+E (F = 0.0141, p<0.05), PE+H (F = 0.022, p<0.05) and PE+E+H (F = 0.0057, p<0.01) compared to PE group. Figure 1b showed a significant difference (ANOVA results) with a value of F<0.0001 in SGPT value. A significant difference also was observed in the control and PE (F<0.0001), PE+E (F<0.0001), PE+H (F<0.0001) and PE+E+H (F = 0.0032, p<0.01) compared to the PE group. Figure 1c showed a significant difference (ANOVA results) with a value of

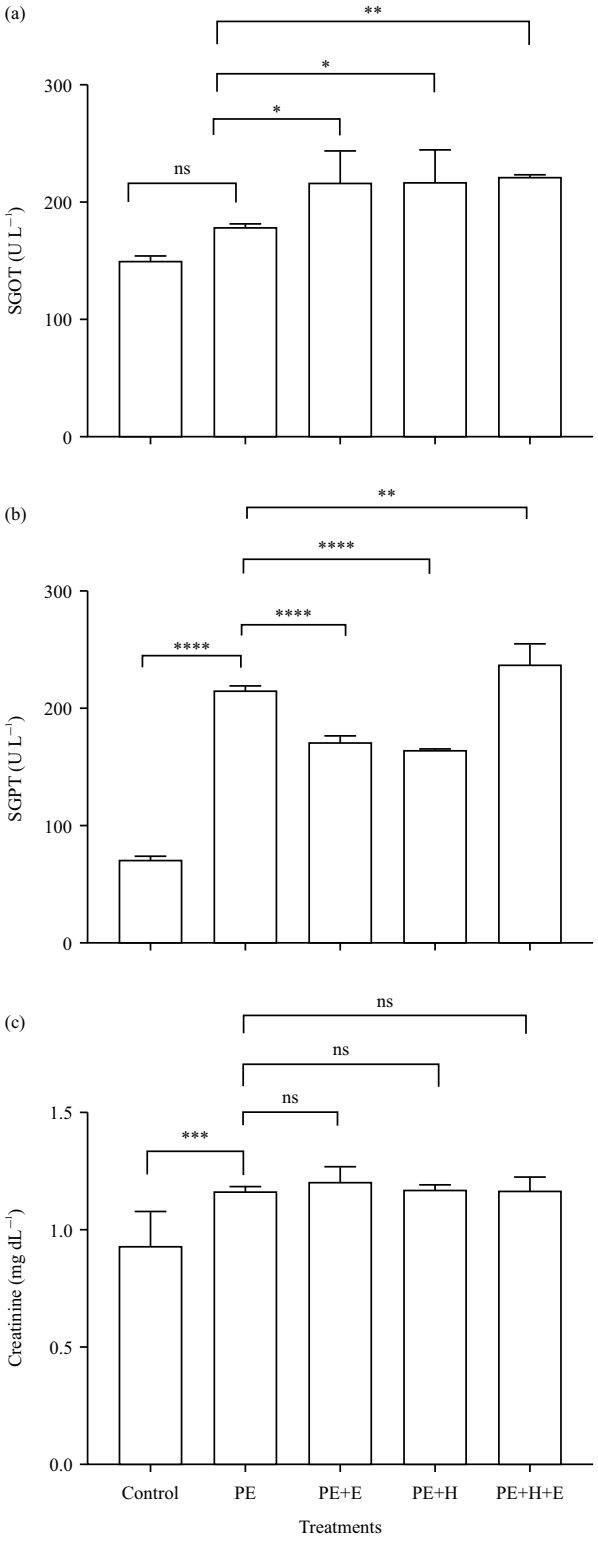

Fig. 1(a-c): (a) SGOT, (b) SGPT and (c) Creatinine
Control: Pregnant rats without treatment, PE: Preeclamptic rats, PE+E: PE rats after giving EVOO, PE+H: PE rats after giving nano herbal haramonting, PE+E+H: PE rats after giving EVOO and nano herbal haramonting, *p<0.05, **p<0.01, ***p<0.001 ****p<0.0001, ns: p>0.05

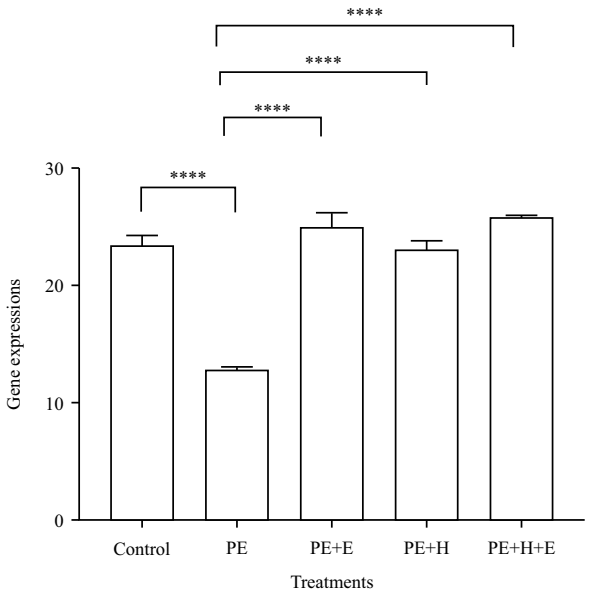

Fig. 2: HSP-70 expressions

Control: Pregnant rats without treatment, PE: Preeclamptic rats, PE+E: PE rats after giving EVOO, PE+H: PE rats after giving nano herbal haramonting, PE+E+H: PE rats after giving EVOO and nano herbal haramonting, ****p<0.0001

F<0.0001 in creatinine value. A significant difference also was observed in the control and PE (F = 0.0009, p<0.001) but an insignificant was observed in the PE+E (F = 0.9921, p>0.05), PE+H (F = 0.998, p>0.05) and PE+E+H (F>0999, p>0.05) compared to PE group. Based on Fig. 1, the value of SGOT, SGPT and creatinine can affect in preeclamptic rats.

HSP-70 expressions: A significant difference (ANOVA results) with a value of F<0.0001 in HSP-70 expressions (Fig. 2). A significant difference also was observed in all groups with the same value (p<0.0001). Based on Fig. 2, HSP-70 expressions affect in preeclamptic rats after given this herbal.

Fetus and placental weight: A significant difference (ANOVA results) with a value of F = 0.0074 (p<0.01) in fetus weight (Fig. 3a). A significant difference was observed in the control and PE (F = 0.0040, p<0.01) but an insignificant was found in the PE+E (F = 0.7333, p>0.05), PE+H (F = 0.5680, p>0.05) and PE+E+H (F = 0.8120, p>0.05) compared to PE group. An insignificant difference (ANOVA results) with a value of F = 0.3629 (p>0.05) in placental weight (Fig. 3b).

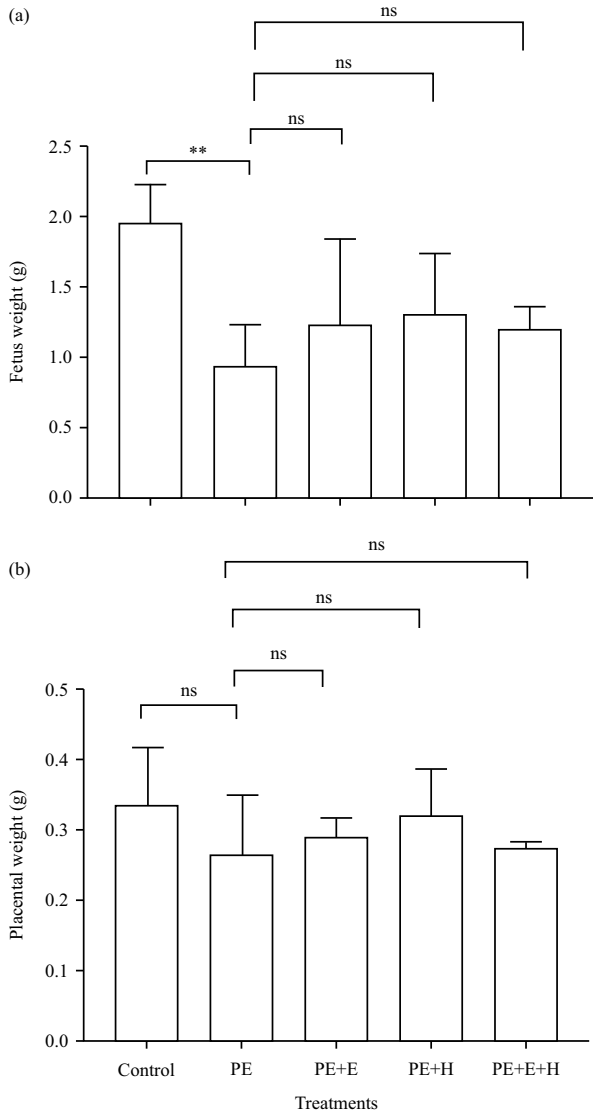

Fig. 3(a-b): (a) Fetus weight and (b) Placental weight

Control: Pregnant rats without treatment, PE: Preeclamtic rats, PE+E: PE rats after giving EVOO, PE+H: PE rats after giving nano herbal haramonting, PE+E+H: PE rats after giving EVOO and nano herbal haramonting, **p<0.01, ns: p>0.05

DISCUSSION

SGOT, SGPT and creatinine are elevated in PE rats (Fig. 1). In addition to the parameters of systolic and diastole blood pressure, the levels of SGOT, SGPT and creatinine can be used as biomarkers of preeclampsia. Because the levels are increased in preeclamptic rats. Significant differences were also found in giving EVOO and haramonting. Both of these herbs reduced SGPT levels but increased SGOT and creatinine levels (Fig. 1). The value of SGOT, SGPT and creatinine can

affect in preeclamptic rats. SGPT and SGOT are excreted from hepatocyte cells when there were cells liver damage[24,25]. The liver is a target organ of toxic substances through the digestive system, then absorbed and carried by portal veins to the liver. The SGOT will increase due to stress on hepatocyte cells and these cells are located between sinusoids filled with blood and bile ducts[4,18]. PE patients usually experience abnormal kidney function, tissue damage, acidosis and increased enzyme activity, xanthine oxidase/dehydrogenase. The administration of haramonting and EVOO increased the levels of SGOT and SGPT in preeclamptic rats (Fig. 1a-b). Measurement of creatinine levels is a biomarker of kidney damage because both of these substances were filtered by the renal glomerulus. Increased creatinine can occur due to dehydration, dietary protein and oxidative stress which can occur due to a diet high in creatine (protein), shock and urinary tract obstruction[9,26]. The increase in creatinine with 70% ethanol extract of haramonting leaves is caused by damage to kidney epithelial cells due to iron-induced free oxidant and ischemia due to heme pigment induced vasoconstriction and causes a decrease in NO availability[27]. However, the increase in creatinine levels in the treatment of haramonting and EVOO were not significant compared to PE (Fig. 1c).

Depressed, regulated placental ischemic and oxidative can affect HSP70 levels. HSP protein will affect and repair damaged cells and anti-apoptosis proteins. Giving antioxidants and tocopherols (vitamin E) to EVOO can increase the production of HSP-70[9,28]. HSP70 levels were increased with EVOO and haramonting in PE rats (Fig. 2). The use of EVOO is useful for supplementing various other antioxidants, such as Vitamin C. Besides tocopherol, EVOO also has components such as phenolics and carotene, which have antimicrobial, antioxidant and anti-inflammatory properties[29]. Haramonting has exceptional antioxidant activity on radical activity, inhibit lipid peroxidation activity and superoxide radical and hydroxyl radical anion activity. This plant extract has flavonoids which can increase SOD activity and GSH-Px activity and reduce MDA levels[30]. The cause of the pathogenesis of preeclampsia is due to an imbalance between antioxidants and Reactive Oxygen Species (ROS) in the placenta and the circulatory system from mother to fetus. Oxidative stress causes disruption of embryonic cells and inflammatory reactions in the mother. ROS will promote lipid oxidation and induce heme oxygenase 1 (HO-1) and HSP70. Here, the role of HSP70 as a second line of defense in systems with antioxidant function is needed. The strongest antioxidants in nano herbal haramonting can affect the activity of HSP70 in the defense system[31]. However, HSP70 levels and some biomarkers of preeclampsia may decrease in PE patients (the results are not as expected), this is due to genetic variation, stability and extracellular space in the cells[6].

Based on Fig. 3, PE rats affect fetus weight. The administration of nano herbal haramonting and EVOO affected in fetus weight, but an insignificantly was found in the difference of both. Haramonting and EVOO also did not affect the weight of the placenta. Disorders of the placenta and fetus cause the release of pro-inflammatory signals (inflammation) which play a key role in various physiological processes, including growth, embryonic development, increased inflammation and angiogenesis (Fig. 3). The placenta requires compounds that will increase trophoblast invasion, spiral arterioles remodeling, increase of placental perfusion and reduce ROS formation in placental tissue. Thereby reducing the level of oxidative stress in the placenta of PE[32].

The implication of the research is the examination of HSP-70 in pregnancy required to get a healthy pregnancy. Nano herbal haramonting can be applied as a drug development consideration in the health of the pregnancy. Further research such as HSP-90, HIF and SFlt-1/PlGF gene expression analysis to the blood of pregnancy after administration of nano herbal haramonting is highly recommended because the limit of this study only to the role of HSP-70.

CONCLUSION

The administration of nano herbal haramonting and EVOO can increase the value of HSP-70 in preeclampsia rats ($p<0.0001$) so that it can reduce lipid peroxidation. Although an insignificant was found in the placental weight. Nanoherbal haramonting can be promising a preeclampsia drug in the future.

SIGNIFICANCE STATEMENT

This study discovers the possible effect of nano herbal haramonting and EVOO combination that can be beneficial for decreased preeclampsia rats. This study will help researchers to uncover that this herb in nanosize may be beneficial in HSP-70 levels in preeclampsia. Because HSP-70 levels are also biomarkers of preeclampsia and affected the anti-apoptosis of damaged cells in the placenta. Thus, a new theory on these herbal combinations and possibly other combinations, may be arrived at.

REFERENCES

1. Eggers, D.K., W.J. Welch and W.J. Hansen, 1997. Complexes between nascent polypeptides and their molecular chaperones in the cytosol of mammalian cells. Mol. Bio. Cell, 8: 1559-1573.
2. Hunter-Lavin, C., E.L. Davies, M.M. Bacelar, M.J. Marshall, S.M. Andrew and J.H.Williams, 2004. Hsp70 release from peripheral blood mononuclear cells. Biochem. Biophys. Res. Commun., 324: 511-517.
3. Molvarec, A., L. Tamasi, G. Losonczy, K. Madach, Z. Prohaszka and J.J.R. Rigo, 2010. Circulating heat shock protein 70 (HSPA1A) in normal and pathological pregnancies. Cell Stress and Chaperones, 15: 237-247.
4. Situmorang, P.C., S. Ilyas and S. Hutahaean, 2019. Study of combination of nanoherbal andaliman (*Zanthoxylum acanthopodium*) and Extra Virgin Olive Oil (EVOO) effects in the expression of Malondialdehyde (MDA), Heat Shock Protein-70 (HSP70) and placental histology of preeclamptic rats. Pharm. Sci., 25: 205-220.
5. Molvarec, A., A. Szarka, S. Walentin, G. Bekő, I. Karádi, Z. Prohászka and J. Rigó, 2011. Serum heat shock protein 70 levels in relation to circulating cytokines, chemokines, adhesion molecules and angiogenic factors in women with preeclampsia. Clin. Chim. Acta, 412: 1957-1962.
6. Vo, T.S. and D.H. Ngo, 2019. The health beneficial properties of *Rhodomyrtus tomentosa* as potential functional food. Biomolecules, Vol. 9, No. 2. 10.3390/biom9020076.
7. Na-Phatthalung, P., M. Teles, S.P. Voravuthikunchai, L. Tort and C. Fierro-Castro, 2018. Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol. Biochem., 44: 543-555.
8. Lavanya, G., S.P. Voravuthikunchai and N.H. Towatana, 2012. Acetone extract from *Rhodomyrtus tomentosa*: A potent natural antioxidant. Evidence-Based Complement. Altern. Med., Vol. 2012. 10.1155/2012/535479.
9. Ilyas, S., S. Hutahaean and D.E. Irianti, 2018. Transforming growth factor expression (TGF-β) correlate with serum level of malondialdehyde (MDA) after EVOO administration in preclinical rat models of preeclampsia. IOP Conf. Ser.: Earth Environ. Sci., Vol. 130. 10.1088/1755-1315/130/1/012048.
10. Roland-Zejly, L., V. Moisan, I. St-Pierre and J.F. Bilodeau, 2011. Altered placental glutathione peroxidase mRNA expression in preeclampsia according to the presence or absence of labor. Placenta, 32: 161-167.
11. Padmini, E., S. Lavanya and V. Uthra, 2009. Preeclamptic placental stress and over expression of mitochondrial HSP70. Clin. Chem. Lab. Med., 47: 1073-1080.
12. Barut, F., A. Barut, B.D. Gun, N.O. Kandemir and E. Aktunc *et al*., 2010. Expression of heat shock protein 70 and endothelial nitric oxide synthase in placental tissue of preeclamptic and intrauterine growth-restricted pregnancies. Pathol. Res. Pract., 206: 651-656.
13. Liu, Y., N. Li, L. You, X. Liu, H. Li and X. Wang, 2008. HSP70 is associated with endothelial activation in placental vascular diseases. Mol. Med., 14: 561-566.
14. Servili, M., B. Sordini, S. Esposto, S. Urbani and G. Veneziani *et al*., 2014. Biological activities of phenolic compounds of extra virgin olive oil. Antioxidants, 3: 1-23.
15. Elgebaly, H.A., N.M. Mosa, M. Allach, K.F. El-Massry, A.H. El-Ghorab, A.M. Al Hroob and A.M. Mahmoud, 2018. Olive oil and leaf extract prevent fluoxetine-induced hepatotoxicity by attenuating oxidative stress, inflammation and apoptosis. Biomed. Pharmacother., 98: 446-453.
16. Cárdeno, A., M.K. Magnusson, H. Strid, C.A. de La Lastra, M. Sánchez-Hidalgo and L. Öhman, 2014. The unsaponifiable fraction of extra virgin olive oil promotes apoptosis and attenuates activation and homing properties of T cells from patients with inflammatory bowel disease. Food Chem., 161: 353-360.
17. Situmorang, P.C., S. Ilyas, S. Hutahaean, R. Rosidah and R.D. Manurung, 2020. Acute toxicity test and histological description of organs after giving nano herbal andaliman (*Zanthoxylum acanthopodium*). Rasayan J. Chem., 13: 780-788.
18. Ilyas, S., F. Murdela, S. Hutahaean and P.C. Situmorang, 2019. The effect of haramounting leaf ethanol extract (*Rhodomyrtus tomentosa* (*Aiton*) Hassk.) on the number of leukocyte type and histology of mice pulmo (*Mus musculus* L.) exposed to electronic cigarette. Open Access Maced. J. Med. Sci., 7: 1750-1756.
19. Situmorang, P.C. and S. Ilyas, 2019. Description of kidney histology of *Mus musculus* after giving nano herbal *Rhodomyrtus tomentosa* (Haramounting). Int. J. Ecophysiol., 1: 26-33.
20. Situmorang, P.C., S. Ilyas and S. Hutahaean, 2019. Effect of combination of nano herbal andaliman (*Zanthoxylum acanthopodium* DC.) and Extra Virgin Olive Oil (EVOO) to kidney histology of preeclampsia rats. IOP Conf. Ser.: Earth Environ. Sci., Vol. 305, No. 1. 10.1088/1755-1315/305/1/012081.
21. Situmorang, P.C., S. Ilyas, S. Hutahaean and R. Rosidah, 2019. Effect of nanoherbal andaliman (*Zanthoxylum acanthopodium*) and extra virgin olive oil combination on preeclamptic rats liver histology. Open Access Macedonian J. Med. Sci., 7: 2226-2231.
22. Nurkhasanah, N., M.S. Bachri and N.R. Azis, 2016. Effect of subchronic submission of rosella flower petal extract (*Hibiscus sabdariffa* L.) on sgpt sgot and alpine rates. J. Pharm. Sci. Community, 13: 90-96.

23. Gao, Y., X. Chen, W. Gao, Y. Yang, H. Ma and X. Ren, 2012. A new purification method for enhancing the immunogenicity of heat shock protein 70-peptide complexes. Oncol. Rep., 28: 1977-1983.
24. Teixeira, R.L.D.F., R.G. Morato, P.H. Cabello, L.M.K. Muniz and A.D.S.R. Moreira *et al*., 2011. Genetic polymorphisms of NAT2, CYP2E1 and GST enzymes and the occurrence of antituberculosis drug-induced hepatitis in Brazilian TB patients. Mem. Inst. Oswaldo Cruz, 106: 716-724.
25. Chatuphonprasert, W. and K. Jarukamjorn, 2012. Impact of six fruits-banana, guava, mangosteen, pineapple, ripe mango and ripe papaya-on murine hepatic cytochrome P450 activities. J. Applied Toxicol., 32: 994-1001.
26. Wu, G., 2020. Important roles of dietary taurine, creatine, carnosine, anserine and 4-hydroxyproline in human nutrition and health. Amino Acids, 52: 329-360.
27. Sakthirajan, R., J. Dhanapriya, A. Varghese, K. Saravanakumar and T. Dineshkumar *et al*., 2018. Clinical profile and outcome of pigment-induced nephropathy. Clin. Kidney J., 11: 348-352.
28. Irianti, E., S. ilyas, Rosidah and S. Hutahaean, 2018. HSP70 expression profile in preeclampsia model of pregnant rat (*Rattus norvegicus*) after giving the EVOO. IOP Conf. Ser.: Mater. Sci. Eng., Vol. 180. 10.1088/1757-899X/180/1/012161.
29. Cicerale, S., L.J. Lucas and R.S.J. Keast, 2012. Antimicrobial, antioxidant and anti-inflammatory phenolic activities in extra virgin olive oil. Curr. Opin. Biotechnol., 23: 129-135.
30. Wu, P., G. Ma, N. Li, Q. Deng, Y. Yin and R. Huang, 2015. Investigation of *in vitro* and *in vivo* antioxidant activities of flavonoids rich extract from the berries of *Rhodomyrtus tomentosa* (Ait.) Hassk. Food Chem., 173: 194-202.
31. Ekambaran, P., 2001. HSP70 expression and its role in preeclamptic stress. Indian. J. Biochem. Biophysic., 48: 243-255.
32. Bainbridge, S.A., E.H. Sidle and G.N. Smith, 2005. Direct placental effects of cigarette smoke protect women from pre-eclampsia: The specific roles of carbon monoxide and antioxidant systems in the placenta. Med. Hypotheses, 64: 17-24.