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Mangosteen Peel Ekstracts Decreased NfκB, Sflt-1, Tnf-α, Blood Pressure and Proteinurine in Mouse Model of Preeclampcia

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ABSTRACT

Preeclampcia is hyperdynamic condition of pregnancy that is syndromed by hipertension and proteinurine after 20 weeks prenancy, related with disable of fisiologis adapt that can decrease perfusion of uteroplacenta. This research has purpose to know the potential of manggosteen peel ekstracts (MPE) to decrease NFκB, sFlt-1, TNF-α and clinis syndroma (hipertension and proteinurine). The method to make mouse model of preeclampsia (MMP) was by using anti mouse-Qa2 dosage 40 ng. There were 5 groups, each group consist of 4 mice, the groups were negatif control group, MMP group and 3 MSE groups with various dosage (200 mg/kg, 400 mg/kg and 800 mg/kg). The result was that average HIF-1α, NFκB, sFlt-1, MDA, TNF-α, blood presure and proteinurine MMP group was higher than MPE. The result of analysis proved that dosage 800 mg/kg is the most effective to decrease all of variable, but it was not significant for proteinurine. MRE as the source of super antioxidant can be the scavenger of free radical, induce antioxidant enzim (increasing antioxidant capasity) for reperfution, so it can be antiinflamation and prevent injury.
KEYWORDS: Preeclampcia, hipoperfusi placenta, oxidative stress, endhotelial dysfuction, antioxidant

INTODUCTION

Preeclampcia is hyperdynamic condition of pregnancy that is syndromed by hipertension and proteinurine after 20 weeks prenancy. Preeclampcia incident is the first couse of 6-8 % number morbidity/mortality maternity and fetus in the world[1)]. Preeclampcia is related with disability of fisiologis adaptation that can decrease perfusion of uteroplacenta. Maternity adaption capabilities is influenced by HLA-G as *immunomodulatory*[2)] in embrio implantation process and tolerance to allograft-fetus[3)], and domination of anti *inflammatory*/Th2 cytokine role like IL-4, IL-5 dan IL-10 and decreasing of *inflammatory*/Th1 cytokine like TNF-α, IFN-γ in uterus micro environment for developing of normal placenta[4, 5)].

Decreasing of placental perfusion in the first trimester will induce transcription factor HIF-1α[2, 6)]. HIF-1α correlated with the increase in cytokines TNF-α through NFkB and improvement of antiangiogenic molecules sVEGFR-1 or sFlt-1 which suppress angiogenesis and inhibit the vascularization of the placenta[7, 8]. Poor placental perfusion will activate free radicals. Excessive free radical without antioxidant can cause oxidative stress which can cause peroxidation toward lipid known by measuring the amount of MDA[1, 9]. Guller in 2009[10)] found that morphology of placenta qualitatively and quantitatively correlated to dysfunction placenta sign caused by inflammation and oxidative stress in preeclampsia. Clinic manifestation (hypertension and proteinurine) of preeclampsia appearance is the sign of endhotelial systemic dysfunction. Complex clinical syndrome can influence all organ system such as haemodinamic, kidney, retina and blood chemical[11)].

Prenatal care is the first important step to prevent preeclampsia by primary (prevent the disease) or secondary care (cure the disease before the identified disease appear) that is almost same as curing the disease. Now a days curing of preeclampsia includes antihypertensive, convulsions control by giving MgSO4, ending the pregnancy or giving birth process.

Recently antioxidant is recommended in potential preventing strategy based of the data that shows that endothelial dysfunction indicates preeclampsia where increased oxidative stress exists, especially in placenta. Many researches used antioxidant approach to cure celluler disorder such as giving combination of vitamin C, E, aspirin and fish oil which can reduce 93% of preeclampcia[12, 13, 14)]. Fhelsi *et al* in 2008[15)] also found a significant association between the intake of vitamin C and vitamin E with MDA. Giving vitamin E can reduce levels of cell membranes isoprostene tropoblas Preeclampcia placenta. This antioxidant supplements were able to inhibit the action of free radicals that can improve the response of the placenta to overcome hypoxia[16)]. Increased lipid peroxidation (F2-

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isoprostan dan MDA), TNF-α and expression NFkC-p50 on the model HUVECs Preeclampcia shown to be reduced by antioxidants[17].

The characteristic of mangosteen rind antioxidant is higher than vitamin E and vitamin C[18]. Some researches of mangosteen rind extract by *invitro showed its ability as an antioxidant*[18-23] . The antioxidant characteristic of mangosteen rind extract comes from xanthone compound, by the bigest component are *α-mangosteen,* and *γ-mangosteen*[24]. While by invivo research especially for the use of mangosteen peel extract to preeclampsia has not been exist.

Scientific studies put mangosteen as the first rank in the world as the fruit which contain antioxidant especially from its rind and seed. The characteristic of mangosteen rind antioxidant is higher than vitamin E and vitamin C[24]. Some researches of mangosteen peel extract *in vitro showed its ability as an antioxidant*[24-29]. Antioxidant properties of mangosteen peel extract compounds derived from xanthone, the largest component is α-mangostin, and γ-mangostin[24]. While research in vivo especially for the use of mangosteen peel extract to preeclampsia has not been exist.

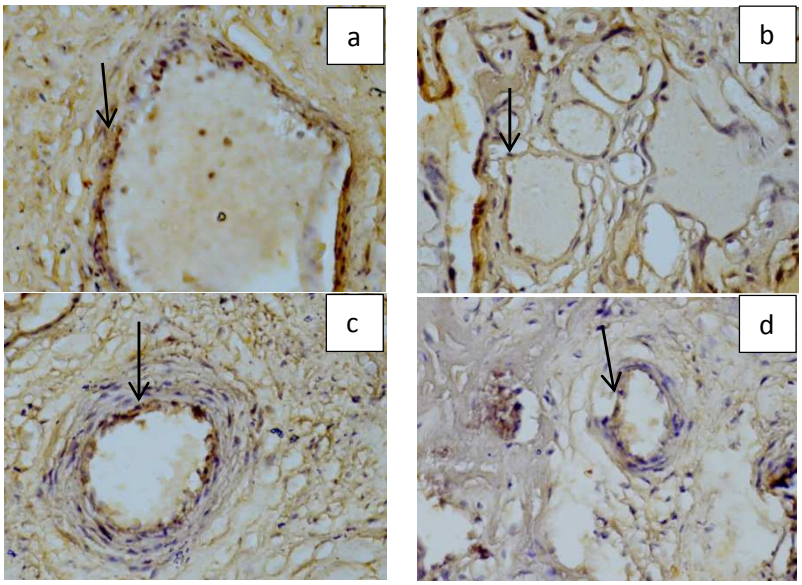
Pathomechanism of preeclampcia that happens in the begining of pregnancy, where oxidative stress increased, it is posible to be cured by antioxidant from mangosteen peel extract so it can prevent or protect membran cell from free radical that can damage it. This research is conducted to prove that mangosteen peel extract can lower both HIF-1α, NFκB, sFlt-1, MDA, TNF-α and preeclampcia clinical syndromes (hypertension and proteinurine) in mouse model preeclampcia.

METHODS

The twenty pregnant mice divided into five groups consist of control group/KK (-), MMP and three groups of MPE with various dosage (200, 400 and 800 miligram/kilogram (mg/kg). MMP injected with anti Qa2 dose 40 ng at the age of 5 day of pregnancy and the treatment group was given a mangosteen peel extract/xanthones in accordance dosage in their 6th until 11th day of pregnancy. At the age of 6 until 11 days of pregnancy (6 days in a row). In MPE groups the mice are by sonde MRE based on the dosage in their 6th until 11th day of pregnancy. In 12th the mice are put in metabolic cage in order to collect their urine for 24 hours and at the 13th day their blood pressure is measured and then they are dissected to take their blood and placenta. Then TNF-α, sFlt-1 dan NFκB are checked by using immunohistokimia method on placenta net [10, 30].

RESULTS

1. Identification HIF-1α on MPE Groups with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP



Picture 1. Differences in expression HIF-1α on (a) the MMP group, (b) the group of MPE dose of 200, (c) group MPE dose of 400 and (d) group MPE dose of 800 mg/kg/day. Seem HIF-1α increased expression of MMP group than the group MPE various doses

2. Identification NFκB on MPE Group with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP

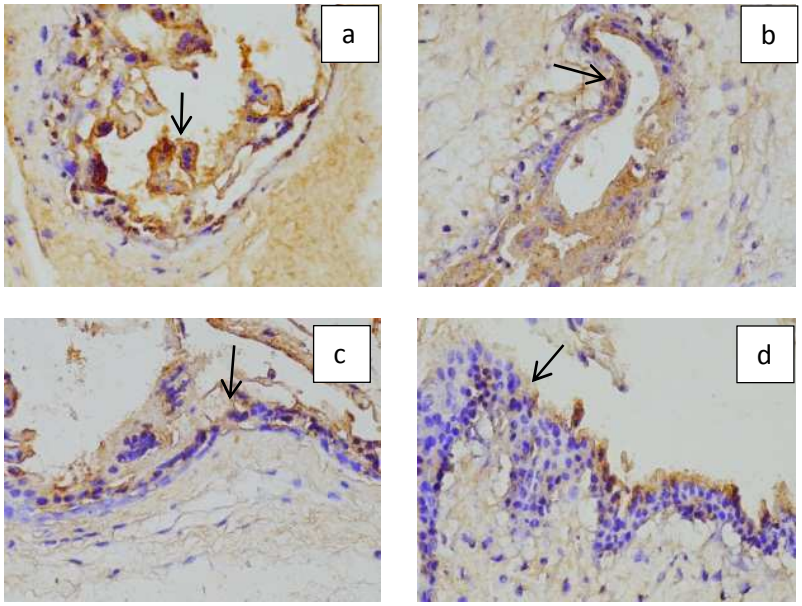


Figure 2. Differences in expression NFκB on (a) the MMP group, (b) the group EKM dose of 200, (c) group MPE dose of 400 and (d) group MPE dose of 800 mg/ kg/day. Seem NFκB increased expression of MMP group than the group MPE various doses

3. Identification of sFlt-1 in Group MPE with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP

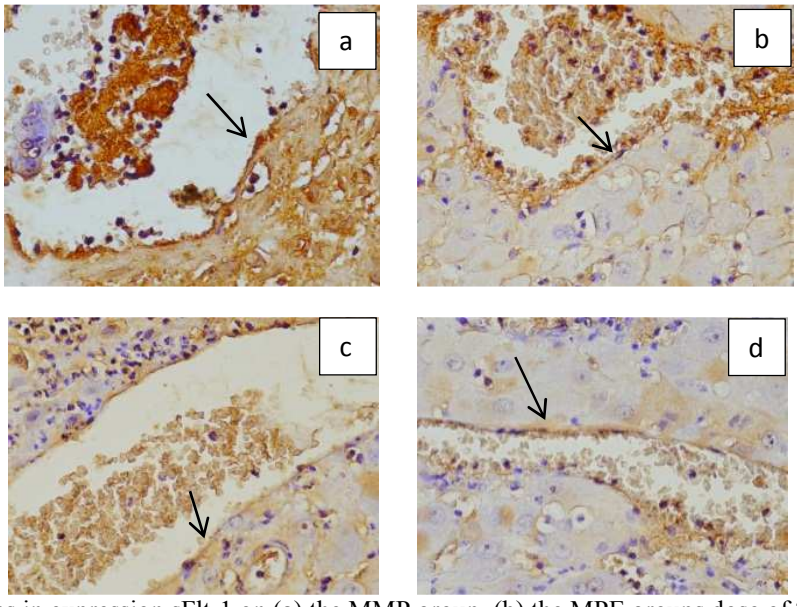


Figure 3. Differences in expression sFlt-1 on (a) the MMP group, (b) the MPE groups dose of 200, (c) group MPE dose of 400 and (d) group MPE dose of 800 mg/kg/day. Seem sFlt-1 increased expression of MMP group than the group MPE various doses

4. Identification MDA on MPE Group with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP

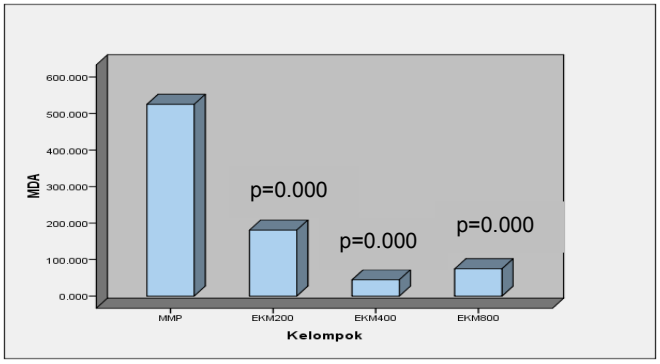


Diagram 1. Differences MDA level in MMP group and MPE groups dose of 200, dose of 400 and (dose of 800 mg/kg/day. Seem increased MDA level of MMP group than the group MPE various doses

5. Identification of TNF-α on MPE Group with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP

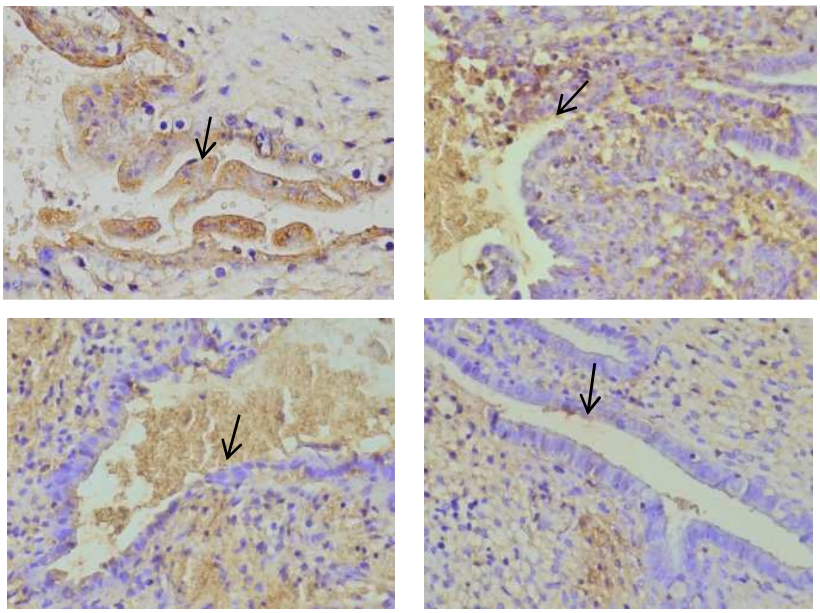
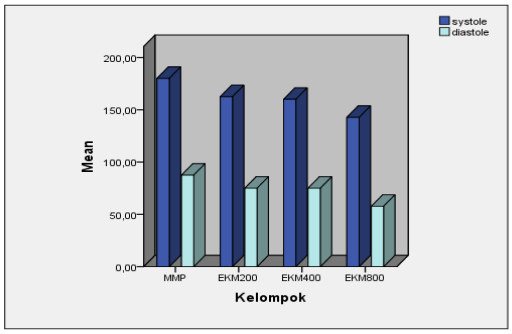


Figure 4. Differences in expression TNF-α on (a) the MMP group, (b) the MPE groups dose of 200, (c) group MPE dose of 400 and (d) group MPE dose of 800 mg/kg/day. Seem TNF-□ increased expression of MMP group than the group MPE various doses

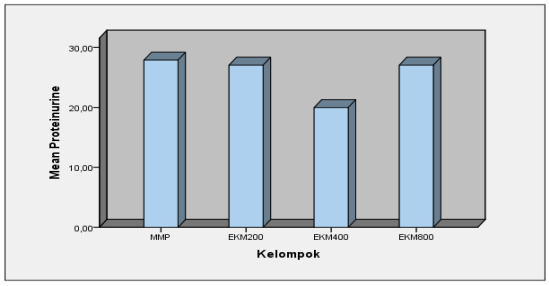
6. Identification of blood pressure in Group MPE with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP



Systole	x	pV
MMP	180±27.08	
MPE-200	162±6.7	0.079
MPE-400	160±6.7	0.047
MPE-800	142±6.7	0.001
Diastole		
MMP	87±15	
MPE-200	75±4.66	0.072
MPE-400	75±4.66	0.072
MPE-800	57±4.66	0.000

Diagram 1. Average distribution blood pressure the MMP group and the group MPE various doses

7. Identification proteinurine on MPE Group with various doses (200; 400 and 800 mg/kg/day) compared to the control group/MMP



	x	pV
MMP	27.88 ± 19.35	
EKM-200	27.05 ± 4.29	0.892
EKM-400	19.97 ± 4.29	0.207
EKM-800	27.05 ± 4.29	0.892

Diagram 2. Average distribution proteinurine the MMP group and the group MPE various doses

DISCUSSION

Patomekanisme the beginning of preeclampsia, there is a failure of cytotrophoblast invasion into the maternal spiral arteries. This will cause a decrease in uteroplacental perfusion, which in turn give rise to a state of hypoxia in the placenta. Conditions of oxygen depletion would trigger the transcription factor, hypoxia-inducible transcription factors (HIF-1α) that stimulate trophoblast, villus cytotrophoblast cells to migrate into cytotrophoblast ekstravillus and invasive cytotrophoblast the vascular endothelium more in the spiral arteries or the so-called process of placentation. Placentation process is needed to ensure an adequate oxygen supply to the developing fetus during pregnancy[31]. Decrease in placental perfusion activates the release of debris placenta derived from sincitiotrofoblas apoptosis, increased cytotoxic factors such as TNF-α and IL-6, increased electron leakage from the respiratory chain due to electron transport in mitochondria is reduced so as to react with molecular oxygen to form free radicals and improve a highly reactive lipid oxidation damage the endothelial cells[32].

In this research it is found the decreasing of average of HIF-1α, NFκB, sFlt-1, MDA, TNF-α expression and clinical syndromes (hypertension and proteinurine) preeclampsia on the MPE groups with various dosages is compared to MMP group. Based on the research by Williams *et al.* (2005)[21] it is found that the function of mangosteen as strong antioxidant in free radical scavenger to protect the damage caused by LDL oxidative by in vitro.

Preeclampsia is a hypertensive disorder of pregnancy caused by abnormal placental function, partly because of chronic hypoxia at the utero-placental junction[33]. Hypoxia inducible factor-1α (HIF-1(alpha)) is a transcription factor transiently expressed as a protein related with ischaemia placental at preeclampsia[34]. The role of HIF prolyl hydroxylases as targets for the salutary effects of metal chelators[35].

The development of the conceptus in the uterus has the potential to improve transcription factors, NFκB. This process occurs during trophoblast elongation which is spaciotemporally associated with conceptus synthesis and release of IL1B concomitant with pregnancy-specific endometrial up-regulation of IL-1 receptors[36]. Of preeclampsia, shallow trophoblast invasion decrease uteroplacental blood flow, triggering systemic inflammation through the NFkB and MAPK pathways[37].

The increasing of sFlt-1 in pregnancy indicated angiogenesis placenta disruption[38]. The decreasing of sFlt-1 after being intervened shown that mangosteen rind extract can improve the function of blood artery. Related to the decreasing of NFκB from the placenta occurred because of the recovery of placenta and the decreasing of perfusion, so that it can grow well. This research is based on Wang *et al* (2002)[3] and Herrera et al. (2014)[20] research, which shows that xanthone has vasorelaxasi blood artery effect and antihypertensi. Otherwise the effect of xanthone protection in reducing oxidative stress exist in α-mangosteen which prevent injury after perfusion which inducting protein oxidation (*protein carbonyl content*), peroxidasi lipid (*malondialdehyde and 4-hydroxynonenal content*), and decreasing of *glutathione*. α-mangostin can be the scavenger of peroxynitrite anion ($ONOO^-$), singlet oxygen (O_2), and superoxide anion (O_2^-). The effect of xanthone toksicity is caused by 3-nitropropionic acid which can weaken the production of ROS. ROS is one of the main source of xanthine/xanthine oxidase, NADPH oxidase and complex I and complex III from elektron mitokondria transport[39, 40]. Chin *et al*, (2008)[5] also reported that if *xanthone* obstruct free radical and oxidative stress, and induct antioxidant enzim such as superoksida dismutase for reperfusion and increase the capacity of miokardium antioxidant. The results are consistent with findings Wibowo, N. et al., (2012)[19] where antioxidant micronutrient supplementation in early pregnancy with low antioxidant status (increased MDA) can reduce the risk of preeclampsia

The research by Irani *at al*., (2010)[41] found that the increasing of TNF-α on preeclampsia compared to the normal pregnancy. This increasing stimulated neutrofil activation and endhotelial dysfunction. The other potential mechanism from TNF-α can influence kidney hemodinamic and artery pressure regulation during pregnancy and change the distribution of endotelin receptor type in kidney[38]. By giving MRE dosage 400 and 800, TNF-α decreased significantly which means that MRE is an anti iinflammation, as reported Librowski *et al*, (2005)[24] that xanthone has strong potential anti inflammation. The strongest anti inflammation activity exists from γ-mangosteen by obstructing syntheses PGE_2 and siklooksigenase (COX)[11].

Increase in sFlt-1 correlated with decreased VEGF and PIGF and cause an increase in blood pressure, proteinuria and glomerular endotheliosis in preeclampsia[38]. In accordance findings Rumiris et al., (2006)[23] that antioxidant supplementation was associated with better maternal and perinatal outcome in pregnant women with low antioxidant status than control supplementation with iron and folate alone.

Different from Magalhaes et al (2011)[18] opinion which said that there is no correlation between antioxidant and preeclampsia, but this research found the correlation of mangosteen rind extract as antioxidant dosage 800 effectively can reduce marker predictor and hypertension on preeclampsia, while MPE dosage 400 and xanthone dosage 800 can decrease preeclampsia marker but it is not correlated to hypertension.

Conclusions

The best dosage (400-800 mg/kg) had decreased ekspresion of protein HIF-1α, NFκB, sFlt-1, MDA, TNF-α, blood presure and proteinurine. In dosage 800 mg/kg it was significant to improve blood presure, but it was not in proteinurine.

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