Annals of Gynecology and Obstetrics Research

Research Article
Published: 03 Nov, 2020

The Role of Andaliman Fruit (*Zanthoxylum acanthoopodium* DC) Extract on the Level of TNF-α, IL-6, Blood Pressure, Mean Arterial Pressure and Proteinuria in Preeclampsia Rat Models

Melvin Nova Gunawanto Barus[1]*, Syafruddin Ilyas[2], Makmur Sitepu[1] and Adang Bachtiar[3]

[1]Department of Obstetrics and Gynecology, University of North Sumatera, Indonesia

[2]Department of Biology, University of North Sumatera, Indonesia

[3]Department of Health Administration and Policy, University of Indonesia, Indonesia

Abstract

Introduction: To determine the effect of andaliman fruit (*Zanthoxylum acanthopodium* DC) on the level of TNF-α, IL-6, blood pressure, Mean Arterial Pressure (MAP), and proteinuria in preeclampsia rat models.

Material and Method: This is an experimental research using 24 laboratory rats (*Rattus norvegicus)* in Biology Laboratory at Faculty of Science, University of North Sumatera. Study subjects were female pregnant rat models (*Rattus norvegicus* sp) that were divided into 4 groups, such as negative Control (C-), positive Control (C+), andaliman extract 200 mg (C1), and andaliman extract 800 mg (C2). Treatment of all samples was carried out simultaneously and during the treatment, it was observed using the Post-test Only Control Group Design method. The examination results were analyzed by using SPSS 25.

Results: Andaliman (*Zanthoxylum acanthopodium DC*) extract had been proven to reduce the level of TNF-α and IL-6 significantly in preeclampsia rat models (P<0.001). Blood pressure and MAP in treatment groups were significantly lower than control groups (p<0.001). Proteinuria was found in all K+, K1, and K2 rat models and shown recovery after 9 days since andaliman extract was given.

Conclusion: This study proves the anti-inflammatory effect of andaliman extract (*Zanthoxylum acantophodium* DC), thus showing a decrease in the levels of proinflammatory cytokines TNF-α and IL6, lower blood pressure, MAP and better proteinuria output.

Keywords: Andaliman; *Zanthoxylum acantophodium* DC; TNF-a; IL-6; Preeclampsia

OPEN ACCESS

Correspondence:
Melvin Nova Gunawanto Barus, Department of Obstetrics and Gynecology, University of North Sumatera, Bunga Lau Street No 17, Kemenangan Tani, Medan, North Sumatera, 20136, Indonesia, Tel: +628126045742; E-mail: melvinngbarus@yahoo.com

Received Date: *05 Oct 2020*
Accepted Date: *29 Oct 2020*
Published Date: *03 Nov 2020*

Citation:
Barus MNG, Ilyas S, Sitepu M, Bachtiar A. The Role of Andaliman Fruit (Zanthoxylum acanthoopodium DC) Extract on the Level of TNF-α, IL-6, Blood Pressure, Mean Arterial Pressure and Proteinuria in Preeclampsia Rat Models. Ann Gynecol Obstetr Res. 2020; 3(1): 1017.

Introduction

Preeclampsia is a condition marked by high blood pressure on the 20th week of gestation with or without proteinuria. Maternal serum levels of IL-6 and TNF-α play a significant role in pathogenesis of preeclampsia [1-4].

Andaliman (*Zanthoxylum acanthopodium DC*) is a species of wild plant known in North Sumatra. On a previous study, administration of andaliman extract appears to inhibit levels of TNF-α and modulate levels of IL-6 compared to the control groups [5,6].

In the present study, we aimed to determine the effect of andaliman extract on the level of TNF-α, IL-6, blood pressure, MAP, and proteinuria in preeclampsia rat models.

Material and Method

This is analytical research with an experimental design that took place in Animal House Laboratory, Faculty of Science, University of North Sumatera from July 2019 until October 2019. The interventions to all samples were given simultaneously and observations were conducted during the interventions by employing *Post Test Only Control Group Design*. Ten weeks old healthy pregnant female rat models *Rattus norvegicus* sp were included in the study. This species of rat models were chosen as study subjects because they have similar genetic characteristic and the ability to adapt

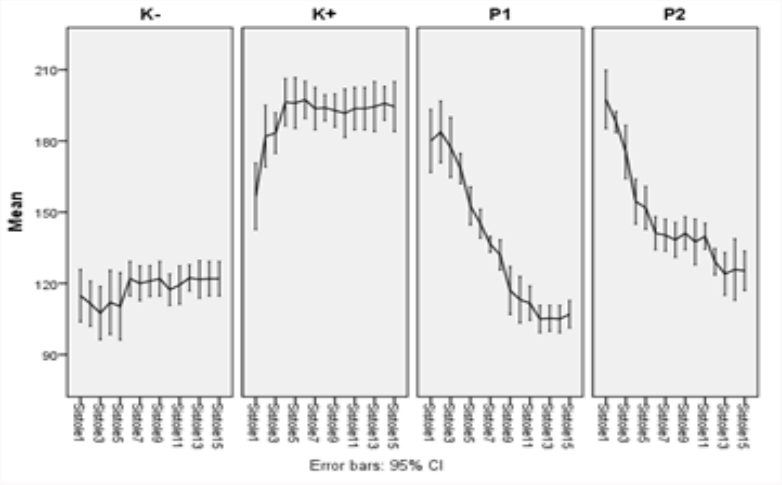

Figure 1: The systolic blood pressure difference between groups.

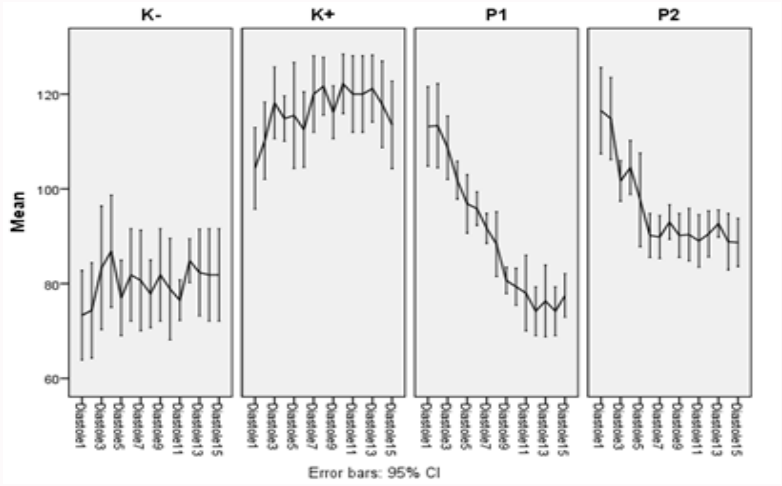

Figure 2: The diastolic blood pressure difference between groups.

like humans. The exclusion criteria were that the blood sample was damaged and cannot be processed and the rat models were dead during the study timeline.

One male and one female rat model were put in the same cage for 1 night. The diagnosis of pregnancy was obtained by the presence of vaginal spermatozoa or vaginal plugs and was counted as day 0 of pregnancy. On the day 0, twenty-four rat models were randomized and divided into 4 groups: Negative Control group (C-) was no intervention rat models, positive Control group (C+) was pregnant rat models which were given 0.5 µg/kg Lipopolysaccharides (LPS) injection during the 5th day of pregnancy but were not given andaliman (*Zanthoxylum acantophodium* DC) extract, treatment group 1 (P1) was pregnant rat models which were given LPS injection and 200 mg/day andaliman (*Zanthoxylum acantophodium* DC) extract for 15 days, treatment group 2 (P2) was pregnant rat models which were given LPS injection and 800 mg/day andaliman (*Zanthoxylum acantophodium* DC) extract for 15 days. During the LPS injection, blood pressure monitoring was done in the morning (8.00 am to 10.00 am) and evaluated every day. Andaliman (*Zanthoxylum acantophodium* DC) extract was given to P1 and P2 groups when there was an increase in systolic blood pressure. Extract was given orally by oral gavage with the recommended maximum volume limit of 5 ml/kg. On the 21st day of pregnancy, termination was done to all study subjects.

Blood sample was taken from heart blood serum and was analyzed by ELISA quantitative method. Blood pressure and MAP were presented in mean and standard deviation, while proteinuria was presented in percentage. To determine the parameter difference (TNF-α, and IL-6) were analyzed by ANOVA if the data were normally distributed, but if the data was not normally distributed, the data will be analyzed by Kruskal Wallis. The difference between both groups was analyzed by Post Hoc analysis.

Results

Changes in systolic and diastolic blood pressure between groups

Both systolic and diastolic blood pressure was in the normal limit for C- group, while for C+, P1 and P2 groups were reduced after the administration of andaliman (*Zanthoxylum acantophodium* DC) extract (Figure 1 and 2). The administration of andaliman (*Zanthoxylum acantophodium* DC) extract had shown the reduction of blood pressure in the treatment group, but remained higher than the C- group. Group P1 had a better prognosis compared to P2 group, because they had lower blood pressure, which nearly approaching the

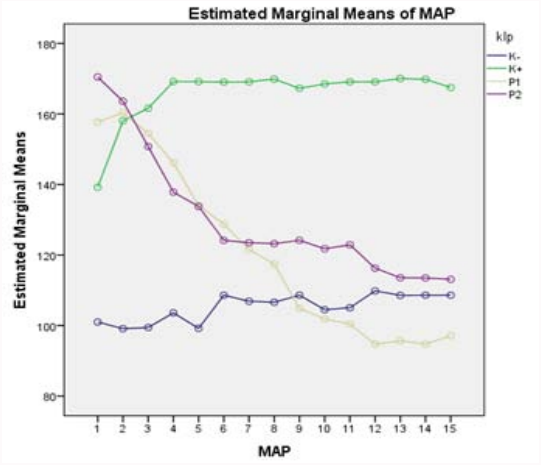

Figure 3: The MAP difference between groups.

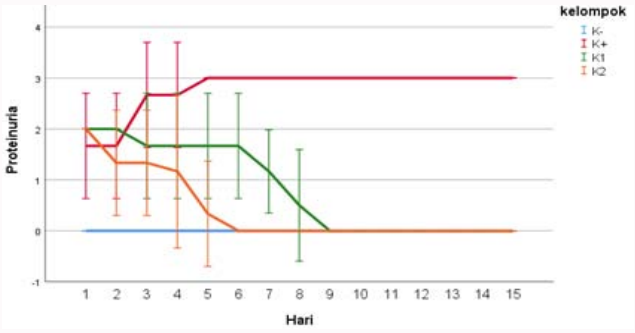

Figure 4: Proteinuria difference between groups.

baseline in C- group.

Changes in MAP between groups after administration of andaliman (*Zanthoxylum acantophodium* DC) extract

There was a significant MAP difference from day 1 to 15 after LPS injection between all groups (p<0.001) (Table 1). This study results showed that MAP in C-group tends to be more stable from day 1 to day 15 after LPS injection, while C+ group had progressive MAP escalation. On the P1 and P2 treatment groups, there was a significant reduction of MAP and nearly approaching the baseline limit, especially on P1 group (Figure 3).

Comparison of proteinuria between groups

Approximately 75% of the rat models had proteinuria, while 25% of the rat models in C- groups did not have proteinuria. Proteinuria +1 was found in 8.3% rat models, while 66.7% of rat models had proteinuria +2. The third day after the administration of andaliman (*Zanthoxylum acantophodium* DC) extract, 25% rat models had negative proteinuria, 25% had proteinuria +1, 33.3% had proteinuria +2 and 16.7% had proteinuria +3. On the 10th day, proteinuria +3 was only found on 25% rat models, while 75% of rat models did not have proteinuria (Figure 4).

Changes in TNF-α level after the administration of andaliman (*Zanthoxylum acantophodium* DC) extract

There was a significant mean TNF-α level difference between treatment groups (P1 and P2) and Control groups (C- and C+), which mean the administration of andaliman (*Zanthoxylum acantophodium* DC) extract can significantly reduce TNF-α levels (p<0.001) and there was no significant difference between treatment groups (p=0.229) (Table 2).

Changes in IL-6 level after the administration of andaliman (*Zanthoxylum acantophodium* DC) extract

The highest mean of IL-6 level was found on C+ group (67.5), while the lowest mean was found on C- group (16.7). There was a significant mean difference of IL-6 after the administration of andaliman (*Zanthoxylum acantophodium* DC) extract between groups (p<0.001), while there was no significant difference between treatment groups on post hoc analysis (p=0.640) (Table 3).

Discussion

This study used 2 different andaliman (*Zanthoxylum acantophodium* DC) doses, which is 200 mg/ml and 800 mg/cc. All the study subjects were given enough foods and treated appropriately, hence the mortality was 0%.

Effects of andaliman (*Zanthoxylum acantophodium* DC) extract on blood pressure

This study results showed there was a significant difference between systolic and diastolic blood pressure on treatment and control groups. P1 treatment group had lower blood pressure, which nearly approaching the baseline compare to P2 treatment group. P2

Table 1: The MAP difference between groups.

(I) klp	(J) klp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
C-	K+	-60.56*	1.837	<0.001	-67.11	-54.01
	P1	-15.44*	1.86	<0.001	-22	-8.89
	P2	-24.96*	2.207	<0.001	-32.18	-17.73
C+	K-	60.56*	1.837	<0.001	54.01	67.11
	P1	45.11*	1.192	<0.001	41.22	49.01
	P2	35.60*	1.682	<0.001	29.73	41.47
P1	K-	15.44*	1.86	<0.001	8.89	22
	K+	-45.11*	1.192	<0.001	-49.01	-41.22
	P2	-9.51*	1.707	0.003	-15.41	-3.62
P2	K-	24.96*	2.207	<0.001	17.73	32.18
	K+	-35.60*	1.682	<0.001	-41.47	-29.73
	P1	9.51*	1.707	0.003	3.62	15.41

Table 2: The difference of TNF-α level between groups.

	Mean[a]	Median	SE	SD	95% CI	Post Hoc[b]				
						p	K-	K+	P1	P2
C-	84.4	85.8	3.3	8.1	76.0-92.9	<0.001		<0.001	0.231	0.032
C+	109.7	111.1	3.3	8.0	101.3-118.1				< 0.001	0.005
P1	90.1	91.4	3.2	7.9	81.8-98.4					0.299
P2	95.1	96.4	3.3	8.0	86.6-103.5					

Table 3: The difference of IL-6 between groups.

	Mean[a]	Median	SE	SD	95% CI	Post Hoc[b]				
						p	K-	K+	P1	P2
C-	16.7	16.7	3.5	8.5	7.8-25.7	<0.001		<0.001	0.673	0.377
C+	67.5	69.9	3.5	8.5	58.6-76.4				<0.001	<0.001
P1	18.8	21.5	3.5	8.5	9.9-27.7					0.640
P2	21.1	23.8	3.4	8.3	12.4-29.9					

[a] Anova; [b] Bonferroni

group also had lower blood pressure compared to C- and C+ groups. The same results were also present of Situmorang's study, which stated that the administration of nano andaliman and olive oil on the 13th to 19th day to preeclampsia rats can significantly reduce systolic blood pressure ($p<0.01$) compared to control group, but there was no significant difference seen on diastolic blood pressure ($p>0.05$) [7].

Wijaya study showed that the reduction of blood pressure on preeclampsia rats which receive andaliman extract probably caused by the anti-inflammation effect of andaliman. Inflammation was associated with the increase of blood pressure due to systemic vasoconstriction which leads to the increasing of vascular resistance and blood pressure [8].

Effects of andaliman (*Zanthoxylum acantophodium* DC) extract on MAP

This study results showed that there was a significant difference in MAP between groups ($p<0.001$). The same results were found on Gong et al. [9] study, which showed that rat models that receive LPS injection on the 5th day will experience significant changes in MAP ($p<0.01$) [9].

Based on Cornelius et al. [10] research on rat models, the increase of MAP probably caused by the increase of pro-inflammatory cytokine such as TNF-α. The increased of TNF-α level caused the decrease in nitric oxide levels, renal plasma flow, and glomerulus filtration rate. In rat models that had uterine hypoperfusion, an increase in TNF-α level was found up to 2 to 3 times the baseline value. The administration of andaliman extract had proven to reduce MAP and suppress pro-inflammatory cytokine such as TNF-α [10,11].

Effects of andaliman (*Zanthoxylum acantophodium* DC) extract on proteinuria

Based on the study results, proteinuria occurred after the LPS injection as preeclampsia induction. This results was also found in other studies, which showed that the increase of proteinuria after 24 h in severe preeclampsia rat models. Based on Xue et al. [12] study, LPS injection was proven to cause preeclampsia by significantly increasing proteinuria on pregnant rat models since 9th day of pregnancy compared to before pregnancy, which the level of proteinuria was 2.02 ± 0.29 mg *vs.* 1.1 ± 0.18 mg ($p<0.01$) [12].

According to Sanjay and Girija, proteinuria is caused by the excessive increase in *soluble Fms - Like Tyrosine Kinase - 1* (sFLT-1) level and decrease of Vascular Endothelial Growth Factor (VEGF) level by podocytes. VEGF deficiency will cause alteration in glomerular endothelial and causes proteinuria, oliguria, and an increase in creatinine. Increased in uric acid can also happen due to the decrease of renal blood flow and angiotensin II, hence uric acid can also be used as a marker for preeclampsia [13].

Effects of andaliman (*Zanthoxylum acantophodium* DC) extract on TNF-α levels

Preeclampsia was assumed related to placentation alteration on early pregnancy, followed by inflammation and progressive endothelial disruption. Various proinflammatory cytokines and mediators, such as TNF-α and IL-6 might have a role in causing preeclampsia and its progression.

TNF-α is a cytokine polypeptide with 17 kD molecular mass and produced by neutrophil, monocyte, and placenta in preeclampsia. TNF-α played a role in trophoblast proliferation regulator and trophoblast differentiation, remodeling of cell adhesion tissue, apoptosis of trophoblast villi, and production of trophoblastic hormones. TNF-α induced oxidative alteration was due to instability of electron flow in mitochondria which caused the release of free radical oxidative and peroxide formation which lead to endothelial cell destruction. TNF-α contributed to abnormal invasion of placenta, endothelial cell destruction, and oxidative stress. TNF-α can also stimulate the production of IL-6, while IL-6 inhibits the release of TNF-α. TNF-α concentration was significantly higher in first and second trimester on women who have a high risk of preeclampsia [14,15].

Based on Zhang et al. [16] study, preeclampsia rat models showed an increase in TNF-α and IL-6 levels on the placenta after the LPS injection and choline administration inhibited the release of cytokine-related to LPS. Another study on pregnant rats which were injected with TNF-α on 14th to 19th day of pregnancy, showed study subjects showed hypertension symptoms and expressed various inflammation cytokines in placenta, aorta, and kidney. Besides that, there was a significant increase in TNF-α level in the preeclampsia rat models [16].

Yanti et al. [6] showed that the administration of andaliman extract can reduce the TNF-α level and actively block mRNA from

TNF-α. During the inflammation process, macrophage played an important role in the immune response *via* protein inflammation production. The inflammation effect of andaliman can suppress the production of a pro-inflammatory cytokine such as TNF-α, hence the TNF-α level can be reduced [6].

Effects of andaliman (*Zanthoxylum acantophodium* DC) extract on IL-6 levels

IL-6 was a glycosylated protein with 21 kDa to 28 kDa molecular weight and 4 stranded structure. IL-6 increases endothelial permeability by changing the shape of cell and rearrange the structure of intracellular actin. IL-6 increases the thromboxane A2 ratio to prostacyclin which occurred in preeclampsia. IL-6 stimulates growth factors derived from thrombocyte which also occurred in preeclampsia. Human endometrium endothelial can phagocyte apoptotic thrombocyte and secrete pro-inflammatory cytokines such as IL-6. This process was believed as one of the mechanisms that contribute to the inflammation response on the placenta in preeclampsia.

This study result showed that IL-6 level on placenta was increased in preeclampsia ($p<0.001$), which meant there was a progressive endothelial destruction and inflammation process. Gong et al. [9] research showed there was an increase of IL-6 level on preeclampsia rat models which received LPS injection compared to control group ($p<0.05$). Zhang et al. [16] observed that preeclampsia symptoms on preeclampsia rat models occurred after receiving 1 g/kg LPS injection and there was increased in IL-6 levels on placenta [9,16].

Conclusion

Andaliman (*Zanthoxylum acanthopodium* DC) is a species of wild plant which has been reported to have anti-inflammatory and antioxidant effects. Andaliman (*Zanthoxylum acanthopodium* DC) contains neolignan, alkaloids, geranyl acetate, amides, and benzoid which have a high potential for the treatment of chronic inflammatory diseases. This research had also proven that andaliman (*Zanthoxylum acanthopodium* DC) extract can reduce blood pressure, MAP, and the proinflammatory cytokine levels which were TNF-α and IL-6. These findings made andaliman (*Zanthoxylum acanthopodium* DC) a novel medicinal plant for the treatment of preeclampsia.

References

1. Lockwood CJ, Yen CF, Basar M, Kayisli UA, Martel M, Buhimschi I, et al. Preeclampsia-related inflammatory cytokines regulate interleukin-6 expression in human decidual cells. Am J Pathol. 2008;172(6):1571-9.
2. Warouw PC, Suparman E, Wagey FW. Karakteristik Penderita Preeklamsia Di Rsup Prof. Dr. Rd Kandou Manado. e-CliniC. 2016;4(1).
3. Qalbi A, Martadiansyah A, Santoso B. Prevalensi Kejadian Preeklampsia Dengan Komplikasi Dan Faktor Risiko Yang Mempengaruhinya Di Rsup Dr. Mohammad Hoesin Palembang (Studi Prevalensi Tahun 2015, 2016, Dan 2017) (Doctoral dissertation, Sriwijaya University). 2018.
4. Li B, Wang H, Zhou G, Zhang J, Su X, Huang Z, et al. VEGF-loaded biomimetic scaffolds: A promising approach to improve angiogenesis and osteogenesis in an ischemic environment. RSC. 2018;7(8):4253-9.
5. Kristanty RE, Suriawati J. The Indonesian Zanthoxylum acanthopodium DC: Chemical and Biological Values. Int J Pharm Tech Res. 2015;8(6):313-21.
6. Yanti PT, NuriasariN, Juliana K. Lemon pepper fruit extract (Zanthoxylum acanthopodium DC) suppresses the expression of inflammatory mediators in lipopolysaccharide-induced macrophages *in vitro*. Ame J Bio Biotech. 2011;7(4):190-5.
7. Situmorang PC, Ilyas S, Hutahaean S, Rosidah R. Effect of nanoherbal andaliman (Zanthoxylum acanthopodium) and extra virgin olive oil combination on preeclamptic rats liver histology. Open access Maced J Med Sci. 2019;7(14):2226.
8. Wijaya CH, Napitupulu FI, Karnady V, Indariani. A review of the bioactivity and flavor properties of the exotic spice "andaliman" (*Zanthoxylum acanthopodium* DC). Food Rev Int. 2019;35(1):1-19.
9. Gong P, Liu M, Hong G, Li Y, Xue P, Zheng M, et al. Curcumin improves LPS-induced preeclampsia-like phenotype in rat by inhibiting the TLR4 signaling pathway. Placenta. 2016;41:45-52.
10. Cornelius DC. Preeclampsia: From inflammation to immunoregulation. Clin Med Insights Blood Disord. 2018;11:1179545X17752325.
11. Amaral LM, Cunningham MW, Cornelius DC, LaMarca B. Preeclampsia: Long-term consequences for vascular health. Vasc Health Risk Manag. 2015;11:403-15.
12. Xue P, Zheng M, Gong P, Lin C, Zhou J, Li Y, et al. Single administration of ultra-low-dose lipopolysaccharide in rat early pregnancy induces TLR4 activation in the placenta contributing to preeclampsia. PLoS One. 2015;10(4):e0124001.
13. Sanjay G, Girija W. Preeclampsia-Eclampsia. J Obstet Gynecolo India. 2014;64(1):4-13.
14. Zhou L, Cheng L, He Y, Gu Y, Wang Y, Wang C. Association of gene polymorphisms of FV, FII, MTHFR, SERPINE1, CTLA4, IL10, and TNFalpha with pre-eclampsia in Chinese women. Inflamm Res. 2016;65(9):717-24.
15. Tavakkol Z, Rahimi HR, Ehteshamfar SM, Ganjali R, Tara F, Moghadam AS. Tumor necrosis factor-α and interleukin-1-β polymorphisms in pre-eclampsia. Iran J Immunol. 2016;13(4):309-16.
16. Zhang M, Bao J, Liu Y, Yang J, Zhang G, Liu H. 40 Gestational choline supplementation prevents inflammatory responses of lipopolysaccharide-induced preeclampsia in pregnant rats: Immune and inflammatory mechanisms. Pregnancy Hypertension: An Int J Women's Cardiovasc Health. 2016;6(3):197.