



Original Article

Calcium supplementation from chicken eggshells to increase nitric oxide levels and control blood pressure in gestational hypertension

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ABSTRACT

Background: Hypertension is one of the highest causes of death in pregnant women. Calcium deficiency can lead to hypertension. Chicken eggshell can be used as a natural calcium supplementation therapy in hypertension.

Purpose: Analyze the effect of chicken eggshell on blood pressure through the increased nitric oxide level in gestational hypertension.

Methods: This is Quasy experiment research with pretest and posttest with control group design. Respondents were ≥ 20 weeks pregnant women with gestational hypertension. Determination of respondents by purposive sampling obtained experimental group ($n=12$) and control group ($n=12$). 1.1 g/day of eggshell flour was given for 14 days.

Result: Mean of systolic and diastolic blood pressure in experimental group decreased 14.25 mmHg or 9.5% ($p=0.000$) and 12.84 mmHg or 13.04% ($p=0.000$) after received chicken eggshell flour for 14 days compared to control group mean of systolic and diastolic blood pressure increased 1.92 mmHg or 1.35% ($p=0.000$) and 0.33 mmHg or 0,34% ($p=0.121$). Nitric oxide level of experimental group increased 8.250 $\mu\text{mol/L}$ or 20.93% ($p=0.020$) and control group decreased 3.083 $\mu\text{mol/L}$ or 7.20% ($p=0.488$).

Conclusion: Chicken eggshell flour affects increasing nitric oxide levels and controls systolic and diastolic blood pressure in gestational hypertension.

INTRODUCTION

94% of total pregnant women's deaths occur in developing countries.¹ Indonesia, one of the developing countries, had an MMR of 305 deaths per 100,000 live births in 2019. That number is far from the target of SDGs in 2030, as much as 70 death per 100,000 live birth.^{2,3} The highest cause of death in Indonesia is hypertension in pregnancy. Hypertension in pregnancy can affect 2-8% of pregnancies; it must be treated because it can threaten both the mother and fetus.⁴ Gestational hypertension is hypertension in pregnancy that occurs after 20 weeks of gestation without being accompanied by protein urine. The incidence rate of gestational hypertension reaches 6-25% and can develop into preeclampsia until it settles into chronic hypertension.⁵ Calcium needs in pregnant women increase in the second and third trimesters of pregnancy. Unmet calcium needs can lead to hypertension in pregnancy. Adequate calcium intake can reduce the risk of

gestational hypertension by up to 45% and also prevent the development of gestational hypertension.^{6,7}

Lowering blood pressure can be done by fulfilling daily calcium intake. Calcium has a vital role in smooth muscle contraction to dilate and prevent hypertension.⁸ All pregnant women are required to get calcium supplementation every day.^{9,10} Calcium is also needed to synthesize nitric oxide, one of the potent vasodilators.¹¹ General calcium supplementation usually given to pregnant women contains calcium lactate with a low elemental calcium content of 13%. Regular consumption of general calcium supplementation every day does not have a significant effect on the blood pressure of pregnant women in previous studies.¹²

Chicken eggshells contain calcium carbonate with an elemental calcium content of 40%.⁹ Bioavailability of chicken eggshells is relatively high compared to available calcium; 90% of the calcium will be absorbed.¹³ Chicken eggshells

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are easily found around us and can be processed into eggshell flour. It can be used as a potential, natural, and current calcium supplementation.¹⁴ Based on examining the composition of eggshell flour, its harmful content is shallow compared to the content in other natural calcium sources.¹⁵ Dangerous pathogens such as salmonella, influenza virus, campylobacter, *Staphylococcus aureus*, and enterobacteria will not be at risk in humans after the eggshell is boiled, so the researchers agree that chicken eggshells are safe to use as calcium supplementation, especially in pregnant women who experience calcium deficiency.¹⁶

Trials of eggshell flour were conducted on hypocalcemia pregnant mice for seven days. The result showed a significant difference in the calcium levels of pregnant mice until they reached the standard limit.¹⁷ The use of eggshell flour has been widely modified as a food mixture to increase calcium levels in foods. However, no studies have examined the benefits of eggshell flour to increased calcium levels and its effect on blood pressure. Previous research is widely researched in nutritional science and focuses on texture changes and calcium levels of food after being mixed with eggshell flour.

Eggshell flour has been shown to increase blood calcium levels in pregnant mice and humans. Fulfillment of calcium levels can prevent and decrease blood pressure in pregnant women. This study was conducted to prove the effectiveness of the use of eggshell flour against the blood pressure and nitric oxide levels of pregnant women with gestational hypertension.

METHOD

Study Design

This is a Quasi-experimental pretest and posttest with a control group design.¹⁸

Setting and Respondents

The research was conducted in the working area of the Public Health Center on Cirebon Regency in May-February 2021. Respondents were pregnant women with gestational hypertension. Samples are calculated using sample size application with reference from previous research.¹⁹ The number of samples was 24 respondents, and they were divided into experimental group (n=12) and control group (n=12) randomly. The inclusion criteria of respondents are pregnant women with gestational age ≥ 20 weeks with blood pressure ≥ 130 mmHg systolic and ≥ 80 mmHg diastolic, willing to obey the schedule of taking drugs and living in the area where the research was conducted. The exclusion was carried out on respondents who consumed pregnancy milk, preeclampsia, diabetes mellitus and had kidney and liver disorders.

Making the Chicken Eggshell Calcium Tablet

Before consumption, eggshells must be processed first into flour. Making eggshell flour is carried out based on the method WHO (2006).¹⁶ The eggshell membrane is removed, washed, and then sterilized by boiling for 30 minutes. After the boiling process is complete, the eggshells are dried at low temperature in the oven, and then the eggshells are crushed into flour. Furthermore, the flour is sifted using a sieve to get a smoother result and then put into capsules.^{13,16}

Experimental Procedure

1.1 grams of eggshell flour put in the capsules was given to 12 respondents in the experimental group daily for 14 days. The control group received 1 gram of general calcium supplementation daily for 14 days. All of the respondents keep consuming 10 mg of nifedipine tablets based on the rules in the Public Health Center.

The Variables, Instrument, and Measurement

The outcomes obtained in this study are mean blood pressure and nitric oxide levels after respondents received treatment for 14 days. On day one, the pretest is done by checking systolic and diastolic blood pressure and taking 3cc of blood to examine the nitric oxide levels. Re-examine of systolic and diastolic blood pressure was done on day 7. The last posttest was done on day 14 by re-checking blood pressure and nitric oxide level. Researchers used an observation sheet to monitor systolic and diastolic blood pressure after obtaining it from the examination results using a sphygmomanometer. The nitric oxide levels were obtained from blood samples analyzed using a microplate reader (ELISA method).

Data Analysis

Analysis used the Friedman and Mann-Whitney tests to determine the effectiveness of the treatment to systolic blood pressure outcome, and Independent T-test was used to determine the outcome of diastolic blood pressure. An Independent t-test analyzed the effectiveness of treatment on nitric oxide levels. Correlation tests are conducted to find the correlation between nitric oxide levels and systolic and diastolic blood pressure.

Ethical Consideration

An ethical license was obtained from the Bioethics Commission of Sultan Agung Islamic University Faculty of Medicine with the registration number: 113/IV/2021/Bioethics Commission.

RESULTS

Table 1 shows that the average age of respondents in this study was over 20 years old. Most of the respondents were multipara. Respondents in the study had to vary BMI scores.

Table 1. Characteristics of Respondent (n=24)

Characteristic	Result
Age	
< 20 years	0 (00.0%)
20-35 years	14 (58.3%)
> 35 years	10 (41.7%)
Parity	
Primigravida	3 (12.5%)
Multipara	21 (87.5%)
BMI	
Normal	9 (37.5%)
Overweight	8 (33.3%)
Obese	7 (29.2%)

The nitric oxide level was measured two times, and the results can be seen in figure 1. The experimental group experienced a significant increase in nitric oxide levels ($p=0.020$) compared to the control group, with insignificant test results ($p=0.488$). The average results of both groups were analyzed using the Independent t-test with a result of $p=0.035$.

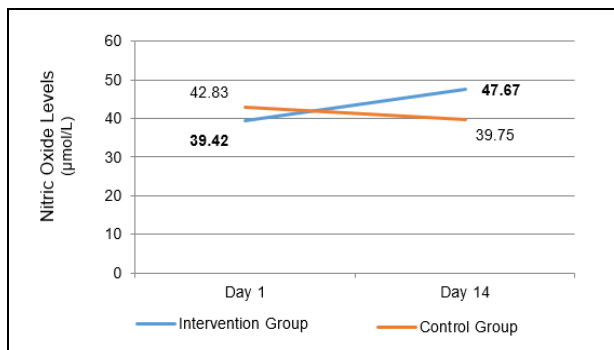
**Figure 1.** The Difference in Nitric Oxide Levels

Figure 2 shows a significant decrease in systolic blood pressure in the experimental group on the seventh and 14th days ($p=0.000$). The control group also decreased on the seventh day, but on the 14th day, there was a slight increase ($p=0.231$). Friedman test results showed that chicken eggshell flour effectively reduced gestational hypertension systolic blood pressure. The results of reducing blood pressure in both groups were analyzed using the Mann-Whitney test with $p=0.053$.

Figure 2 also shows a significant decrease in diastolic blood pressure in the experimental group in each measurement ($p=0.000$). While in the control group, there was a slight increase on the seventh day and a decrease on the 14th day ($p=0.121$). The results showed that chicken eggshell flour effectively lowered diastolic blood pressure. An independent t-test was performed on the results of both groups with $p=0.000$.

Table 2. Correlation between Nitric Oxide Levels with Systolik dan Diastolik Blood Pressure

Variable	Δ Sistolik		Δ Diastolik	
	p-value	r	p-value	r
Δ Nitric Oxide	0.027	- 0.261	0.011	- 0.298

The correlation test results between nitric oxide levels and blood pressure values can be seen in Table 2. The p-value is <0.05 explains a strong correlation between systolic and diastolic blood pressure and nitric oxide levels. The negative values on Pearson correlation results indicate that the increased value of nitric oxide levels may decrease systolic and diastolic blood pressure outcomes in gestational hypertension.

DISCUSSION

Calcium supplementation from eggshell flour can reduce the average systolic and diastolic blood pressure and increase nitric oxide levels in gestational hypertension. Calcium is one of the minerals needed by the body in more significant amounts than the needs of the other mineral elements.²⁰ Calcium levels are determined by the absorption of calcium in the body and parathyroid hormones, calcitonin regulates its balance and vitamin D. Lack of calcium intake will stimulate parathyroid hormone, thus increasing intracellular calcium levels. Muscle cells containing much calcium will bind to troponin, a contractile protein that detaches from its bond with actin and myosin. That interaction causes vasoconstriction in the blood vessels' smooth muscles, leading to hypertension.²¹

Moreover, calcium in the form of serum calcium ions is needed in nitric oxide synthesis, which is a potent vasodilator. It can signal the smooth muscle of blood vessels to dilate. This will help in the process of lowering blood pressure.^{22,23} The increase of free radicals in remodeling the placenta will cause endothelial dysfunction that triggers a decrease in nitric oxide levels. Lack of nitric oxide in the blood circulation of pregnant women has a significant influence on the incidence of hypertension in pregnancy and can develop into preeclampsia. The more severe endothelial damage will reduce nitric oxide production, so the blood pressure continues to increase and worsen.^{11,24} This is supported by previous research that nitric oxide levels are lower in pregnant women with preeclampsia compared to nitric oxide levels in normal pregnancy.²⁴

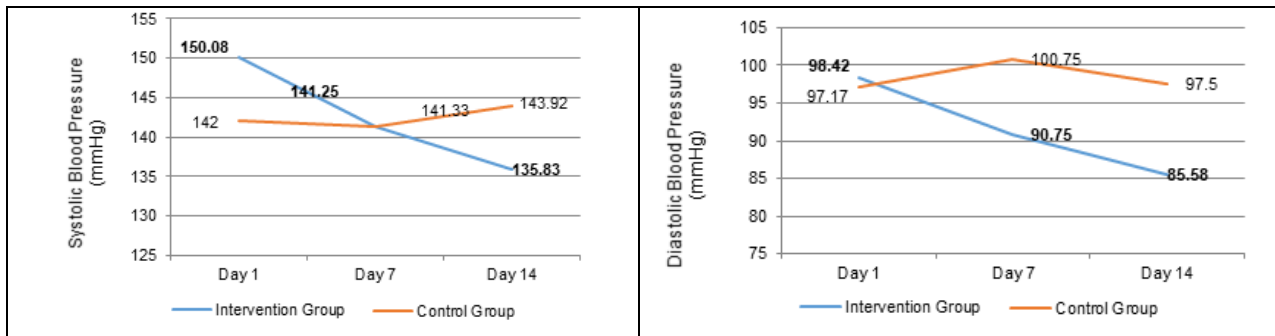


Figure 1. Difference between Systolic and Diastolic Blood Pressure

WHO recommends that all pregnant women consume calcium supplementation as a part of prenatal care to prevent hypertension.⁶ General calcium, usually given, contains calcium lactate with the lowest elemental calcium content of 13%.⁹ Previous study shows that there is no significant difference in blood pressure in pregnant women who routinely and do not routinely consume general calcium supplementation.²⁵

Chicken eggshells are one of the high sources of calcium with calcium carbonate content that has the highest elemental calcium, which is 40%.⁹ Chicken eggshells can be processed into flour using a combined five keys to safer food by WHO. Once processed into flour, chicken eggshells can be safe to consume to increase calcium levels in the body because the threat of pathogens will not be at risk after the chicken eggshell is boiled.¹⁶ Previous research has proven that supplementation of eggshell flour can increase the calcium levels of pregnant mice with hypocalcemia.¹⁷ Absorption process of calcium from chicken eggshells can absorb better in the body because eggshells have a protein matrix that can help the transportation of calcium and is more easily absorbed by the body.²⁶

Respondents who consume chicken eggshell flour capsules do not feel comfortable with the size of the capsules. However, respondents who choose to consume it by mixing it into food or drink say that the texture of eggshell flour tastes like fine sand but does not affect the taste or smell of the food. Many studies have used chicken eggshell as an additional ingredient for making food, such as research that Hassan has done by making biscuits mixed with eggshell flour. There was no significant change in the taste and texture of the biscuits with the addition of 6% eggshell flour.¹³

CONCLUSIONS AND RECOMMENDATION

1.1 grams of eggshell flour supplementations for 14 days effectively decrease systolic and diastolic blood pressure and increase nitric oxide levels in pregnant women with gestational hypertension. Further researchers are expected to be able to examine the other side effects of using eggshell flour. Other than that, chicken eggshell flour can

be consumed by mixing as an addition to certain foods to reduce respondents' discomfort.

REFERENCES

1. WHO. Maternal mortality; <https://www.who.int/news-room/fact-sheets/detail/maternal-mortality>. Accessed January 21, 2021
2. Kemenkes RI. *Profil Kesehatan Indonesia 2018*. Pusat Data dan Informasi Kementerian Kesehatan; 2019.
3. Susiana S. *Angka Kematian Ibu: Faktor Penyebab dan Upaya Penanganannya*. Vol 11. Jakarta Pusat; 2019. doi:9772088235001
4. Saifuddin AB, Wiknjastro H, Saifuddin AB, Rachimhadhi T. *Ilmu Kebidanan Sarwono Prawirohardjo*. IV. (Prawirohardjo S, ed.). Jakarta: PT Bina Pustaka Sarwono Prawirohardjo; 2014.
5. Malha L, Podymow T, August P. *Hypertension in Pregnancy in Hypertension: A Companion to Braunwald's Heart Disease*. Elsevier Inc.; 2018.
6. World Health Organization. *Guideline: Calcium Supplementation in Pregnant Women*; 2013.
7. Imdad A, Bhutta ZA. Effects of Calcium Supplementation During Pregnancy on Maternal, Fetal and Birth Outcomes. *Paediatr Perinat Epidemiol*. 2012;26(1):138–152. doi:10.1111/j.1365-3016.2012.01274.x
8. Kanagal DV, Rajesh A, Rao K, et al. Levels of Serum Calcium and Magnesium in Pre-eclamptic and Normal Pregnancy: A Study from Coastal India. *J Clin Diagnostic Res*. 2014;8(7):1–4. doi:10.7860/JCDR/2014/8872.4537
9. Irwinda R. Peran Kalsium dan Magnesium pada Kehamilan. *MEDICINUS*. 2020;33(1):3–7.
10. World Health Organization. *WHO recommendation: Calcium supplementation during pregnancy for the prevention of pre-eclampsia and its complications*; 2018.
11. Astutik P, Wirjatmadi B, Adriani M. Jurnal Gizi Klinik Indonesia Peranan Kadar Nitrit Oksida (NO) Darah dan Asupan Lemak pada Pasien Hipertensi dan Tidak Hipertensi. 2013;10(02):55–60.

12. Nugroho H, Masturoh SA, Pertiwi RW. Pengaruh Kepatuhan Penggunaan Suplemen Kalsium Terhadap Tekanan Darah pada Pasien Ibu Hamil di Puskesmas Kecamatan Koja Jakarta Utara Tahun 2017. *Jurnal Kesehatan STIKes IMC Bintaro*. 2020;III(1):56–64.
13. Hassan NMM. Chicken Eggshell Powder as Dietary Calcium Source in Biscuits. *World Journal Dairy Food Science*. 2015;10(2):199–206. doi:10.5829/idosi.wjdfs.2015.10.2.1152
14. Chakraborty S, Datta S. Eggshell: An Alternative, Cheap, Bioavailable Source of Calcium in Human Diet. *Research % Reviews: Journal Dairy Science and Technology*. 2019;8(2):25–33.
15. Waheed M, Butt MS, Shehzad A, et al. Eggshell calcium: A Cheap Alternative to Expensive Supplements. *Trends Food Science & Technology*. 2019;91(July):219–230. doi:10.1016/j.tifs.2019.07.021
16. Bartter J, Diffey H, Yeung YH, et al. Use of chicken eggshell to improve dietary calcium intake in rural sub - Saharan Africa. *Wiley Matern Child Nutrition*. 2018;14(February):1–10. doi:10.1111/mcn.12649
17. Safitri IR, Supriyana, Bahiyatun. Effect of Eggshell Flour on Blood Calcium Levels in Pregnant Mice. *Belitung Nurse Journal*. 2017;3(6):791–795.
18. Stratton SJ. Quasi-Experimental Design (Pre-Test and Post-Test Studies) in Prehospital and Disaster Research. *Prehospital and Disaster Medicine*. 2019;34(6):33–34. doi:10.1017/S1049023X19005053
19. Wijayanti, Apriani A. Efektivitas Konsumsi Tablet Kalsium dan Konseling Gizi terhadap Kadar Kalsium Darah bagi Ibu Hamil. *MATERNAL*. 2018;II(4):244–249.
20. Cormick G, Belizán JM. Calcium Intake and Health. *Nutrients*. 2019;11(7):1–16. doi:10.3390/nu11071606
21. Puspita A, Rusdi, Ilyas EI. Kadar Na⁺, K⁺, Cl⁻, dan Kalsium Total Serum Darah serta Hubungannya dengan Tekanan Darah pada Penderita Hipertensi. *Bioma*. 2015;11(1):50. doi:10.21009/bioma11(1).6
22. Gustirini R. Suplementasi Kalsium pada Ibu Hamil untuk Mengurangi Insidensi Preeklampsia di Negara Berkembang. *Jurnal Kebidanan*. 2019;8(2):151–160.
23. Hermann M, Flammer A, Luscher TF. Nitric Oxide in Hypertension. *The Journal Clinical Hypertension*. 2006;8(12): 17–29. doi:10.1111/j.1524-6175.2006.06032.x
24. Ridharakhim H, Sulistyowati S, Respati SH. Perbedaan Kadar Serum Endothelin-1 (ET-1) dan Nitrit Oxide (NO) pada Preeklampsia Early Onset dan Kehamilan Normal. *Universitas Sebelas Maret*. 2014.
25. Febriana E, Rahfiludin MZ, P DR. Hubungan Asupan Natrium, Kalsium Dan Magnesium Dengan Tekanan Darah Pada Ibu Hamil Trimester II Dan III (Studi di Wilayah Kerja Puskesmas Bulu Kabupaten Temanggung). *Jurnal Kesehatan Masyarakat*. 2017;5(4):648–655. doi: 10.14710/jkm.v5i4.18733
26. Daengprok W, Garnjanagoonchorn W, Naivikul O, Pornsinlpatip P, Issigonis K, Mine Y. Chicken Eggshell Matrix Proteins Enhance Calcium Transport in The Human Intestinal Epithelial Cells, Caco-2. *J Agric Food Chem*. 2003;51(20):6056–6061. doi:10.1021/jf034261e