### Hendro Sudjono Yuwono,<sup>1</sup> Fadhli Rajif Tangke,<sup>2</sup> Reni Farenia<sup>3</sup>

<sup>1</sup>Department of Surgery Faculty of Medicine Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, <sup>2</sup>Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia, <sup>3</sup>Department of Biomedical Sciences Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia

### Abstract

**Background:** Calcium bentonite has been known to decrease high blood cholesterol level. This study aimed to explore whether a local calcium bentonite from West Java, Indonesia, had effect on lowering total blood cholesterol level.

**Methods:** A laboratory experimental study was carried out on thirty adult male Wistar rats' at Laboratory animals, Department of Pharmacology and Therapy, Universitas Padjadjaran in 2012. The rats were divided into five groups with six rats in each group, consisting of group I (negative control) with standard diet and distilled water, group II (positive control) with high lipid diet and distilled water, group III (positive control) with high lipid diet and calcium bentonite 0.25 g/rat/day, group V with high lipid diet and calcium bentonite 0.5 g/rat/day. Ezetimibe as was used as this was an inhibitory agent of intestinal cholesterol absorption. Blood cholesterol levels were measured and analyzed using analysis of variance and Tukey's post-hoc test.

**Results:** Oral calcium bentonite at 0.25 g/rat/day and 0.5 g/rat/day significantly decrease total blood cholesterol level relative to the positive control group, 31.68% (p=0.018) and 32.87% (p=0.006) respectively, but its effect is inferior to ezetimibe 62.83% (p=0.000).

**Conclusions:** Local calcium bentonite from West Java, Indonesia, has a significant lowering effect, however, the effect is less comparing to ezetimibe.

Keywords: Blood cholesterol, calcium bentonite, ezetimibe, wistar rat

# Introduction

Consumption of high saturated fat could increase cholesterol level in blood.<sup>1</sup> Several studies have proven that bentonite could decrease high blood cholesterol level.<sup>2-4</sup> Bentonite is a kind of clay which has an adsorptive ability because of its structure.<sup>5</sup> Indonesia contains abundant bentonite in many areas.<sup>6</sup> This study was conducted to analyze the effect of local calcium bentonite (ca-bentonite) on lowering total blood cholesterol level using hypercholesterolemic rats and was compared to ezetimibe which is an inhibitory agent of intestinal cholesterol absorption.<sup>7</sup>

### **Methods**

This experimental method of study was carried out on laboratory animals, at the Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Padjadjaran from 24 October to 13 November 2012, was approved by the Research Ethics Committee, Faculty of Medicine Universitas Padjdajaran. Adult Wistar rats weighting between 200–250 g were used. This study used Ca-bentonite which was a gift from the Indonesia University of Education from excavation in the Karangnunggal area, Tasikmalaya, West Java, Indonesia. Laboratory tests were performed to confirm the accuracy of the ca-bentonite substance at the Chemistry laboratory, Bandung Institute of Technology. The determination of ca-bentonite dose is based on the lethal dose which is more than 5 g/kg of body weight.<sup>8,9</sup> The first dose is 1 g/kg of body weight, 0.25 g/rat/day. The second dose is 2 g/kg of body weight, 0.5 g/rat/day. Conversion dose of ezetimibe (10 mg of daily human dose) to rat dose is 0.18 g/rat/day. The high lipid diet used in this study was composed of 1.5% chicken egg yolk, 10% fat goat, 1% coconut oil and a mixed of standard diet till 100%.10

**Correspondence:** Hendro Sudjono Yuwono, Department of Surgery, Faculty of Medicine, Universitas Padjadjaran/Dr. Hasan Sadikin General Hospital Bandung, Jalan Pasteur 38, Bandung, Indonesia, Email: hsyabc47@gmail.com

Group	I	II	III	IV	v
Mean	82.8	105.0	62.8	79.7	76.3
±SD	9.93	7.74	6.99	16.54	18.79

# **Table 1 Mean of Total Blood Cholesterol Level**

Note: SD=Standard Deviation

After seven days of adaptation time, in each group, six rats were randomly given intervention for fourteen days. Group I (negative control) was given standard diet and distilled water, group II (positive control) was given high lipid diet and distilled water, group III was given high lipid diet and ezetimibe 0.18 g/rat/day, group IV was given high lipid diet and ca-bentonite 0.25 g/rat/day, and group V was given high lipid diet and ca-bentonite 0.5 g/rat/day. All diets were given ad libitum while ezetimibe and ca-bentonite were given once a day orally. Blood was taken of the distal part of the tail, and then the total blood cholesterol level was measured using an enzymatic reaction and photometer after centrifugation. Normality and homogeneity of the data were tested using the Saphiro-Wilk and Levene test respectively, and then continued with analysis

of variance (ANOVA) and Tukey's post-hoc test.

#### **Results**

The data were normal and homogenous. Group II (positive control) had an increase of total blood cholesterol level relative to group I (negative control). The intervention groups (group III, IV, V) had a decrease of total blood cholesterol level relative to group II. Group III had a decrease as much as 62.83% (p=0.000), group IV as much as 31.68% (p=0.018), and group V as much as 32.87% (p=0.006). Statistic analysis using ANOVA and Tukey's post-hoc test showed significant difference of the total blood cholesterol level between groups (Table 1 and Figure 1).



Note: Group I = negative control Group II = positive control Group III = ezetimibe 0.18 g Group IV = ca-bentonite 0.25 g Group V = ca-bentonite 0.5 g

Figure 1 Mean of Total Blood Cholesterol Level

# Discussion

Montmorillonite as a main part of bentonite contains three main layers, which comprise of two layers of silica tetrahedral and a layer of octahedral aluminum. It has an adsorptive ability due to an imbalance of electric charges between the ion.<sup>5</sup> Bentonite clay also has strong absorptive affinity with organic and inorganic elements.<sup>11</sup> The study results were parallel to in vivo and in vitro studies by Gershkovich et al.<sup>2</sup> that show a decrease in the blood cholesterol level. It is associated with the redistribution and sedimentation of cholesterol as well as an interfering of micelles formation which is important in intestinal cholesterol absorption.<sup>2,4</sup> Likewise a twelve weeks study by Sivak et al.<sup>3</sup> also shows the same results. Humans have used bentonite clay for various intoxication disorders without causing serious side effects when consumed in non-excessive doses. Moosavi<sup>12</sup> (2017) reveals that there are 100 scientific articles from the 2500 articles published by PubMed, showing the influence of the clay on various organs of the human body.12 The results do not cause side effects when used with the right dose.

In several regions of Indonesia, such as in Jatiwangi (West Java) the clay is named Hampo, and in Tuban (East Java) it is called Ampo. In those regions, for decades, there is a tradition of consuming clay as a snack without reports of side effects and they consider it as a health food. In that area, the clay is consumed by children, adults, and pregnant women. Our results strengthened the truth of clay which was considered as food and traditional medicine that was useful for the health of the human body; thus encouraging further research so that it could be used officially as a drug. There are many advantages of using natural medicine, which do not need preservatives, while ezetimibe is a synthetic that requires a chemical preservative, and is much more expensive.

In conclusion, we have found that local ca-bentonite from West Java, Indonesia, can decrease the total blood cholesterol level in hypercholesterolemic Wistar rats but its effect is less effective than ezetimibe. Further study is needed in clinical trials in order to utilize bentonite to be used as an alternative therapy.

# References

1. Michelle Mcguire PD, Beerman KA. Nutritional Sciences: from fundamentals to Food. 3<sup>rd</sup> ed. Belmont: Cengage Learning; 2011.

- 2. Gershkovich P, Darlington J, Sivak O, Constantinides PP, Wasan KM. Inhibition of intestinal absorption of cholesterol by surface-modified nanostructured aluminosilicate compounds. J Pharm Sci. 2009;98(7):2390–400.
- 3. Sivak O, Darlington J, Gershkovich P, Constantinides PP, Wasan KM. Protonated nanostructured aluminosilicate (NSAS) reduces plasma cholesterol concentrations and atherosclerotic lesions in Apolipoprotein E deficient mice fed a high cholesterol and high fat diet. Lipids health dis. 2009;8(1):30.
- Gershkovich P, Sivak O, Contreras-Whitney S, Darlington JW, Wasan KM. Assessment of cholesterol absorption inhibitors nanostructured aluminosilicate and cholestyramine using in vitro lipolysis model. J Pharm Sci. 2012;101(1):291–300.
- 5. Supeno M. Bentonit Terpilar dan Aplikasi. Medan: USU press; 2009.
- Pusat Penelitian dan Pengembangan Teknologi Mineral dan Batubara. Informasi Mineral dan Batubara. Kelompok Program Teknologi Informasi Pertambangan; 2005 [cited 2012 May 4]; Available at: http:// www.tekmira.esdm.go.id/data/Bentonit/ ulasan.asp?xdir=Bentonit&commId=8&co mm=Bentonit.
- Katzung B, Masters S. Basic and Clinical Pharmacology. 12<sup>th</sup> ed. San Fransisco: McGraw-Hill; 2011.
- 8. European Chemical Bureau. IUCLID Dataset-Bentonite. Livingston: European Commission; 2000.
- 9. American Colloid Company. Volclay HPM20. Arlington Heights: Material Safety Data Sheet; 2008.
- 10. Hardiningsih R, Nurhidayat N. The effect of consuming the hypercholesterolemia rationed food to the body weight of white rats wistar with administration of lactic acid bacteria. Biodiversitas. 2006;7(2):127–30.
- 11. Eba F, Gueu S, Eya'A-Mvongbote A, Ondo JA, Yao BK, Ndong Nlo J, Biboutou RK. Evaluation of the absorption capacity of the natural clay from Bikougou (Gabon) to remove Mn (II) from aqueous solution. International Journal of Engineering Science and Technology. 2010;2(10):5001-16.
- 12. Moosavi M. Bentonite Clay as a Natural Remedy: a brief review. Iran J Public Health. 2017; 46(9):1176-83