

Effect of Bean Extract (*Phaseolus vulgaris* L) to LDL and Ox-LDL and Relation of Ox-LDL on LOX-1 Gene Polymorphism 3'UTR188CT

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Abstract

Aim: To find out whether bean extract has effects on levels of LDL and Ox-LDL and relation of Ox-LDL on LOX-1 gene polymorphism 3'UTR188CT.

Method: This research is true randomized experiment with a pretest - posttest design and control group. Samples were 35 subjects divided into 4 groups. Laboratory experiments using bean extract test affects LDL and plasma levels of Ox-LDL by Enzyme-linked Immunosorbent Assay (ELISA), test LOX-1 gene polymorphism 3'UTR188CT with restriction enzyme. To evaluate effect of bean extract to LDL and Ox-LDL level were compared with unpaired t-test. To evaluate difference of Ox-LDL level on LOX-1 gene polymorphism 3'UTR188CT using ANOVA. **Results:** There was a decrease of LDL found in all treatment groups and elevated levels of Ox-LDL. TT genotype had higher levels of plasma Ox-LDL both before ($49,9 \pm 8,3$ vs. CC $39,9 \pm 19,4$ and CT $42,8 \pm 61,2$, p .648) and after treatment ($60,3 \pm 5,7$ vs. CC $55,93 \pm 12,6$ and CT $53,9 \pm 10,3$, p .630) than other genotype. **Conclusion:** Bean extract can lower LDL cholesterol although it can also increased plasma level of Ox-LDL. TT genotype had higher levels of plasma Ox-LDL than other genotypes. Require further study to other antioxidants may have a beneficial effect by lowering the levels of Ox-LDL.

Keywords : Bean extract, LDL, Ox-LDL level, genotype, atherosclerosis

1. Introduction

Atherosclerosis is a chronic inflammation of the blood vessels, which would lead to the emergence of atherom plaque, a focal lesion located in the intima of the blood vessels. Cardiovascular disease is the leading cause of death in most developed countries. Most deaths from cardiovascular disease can be prevented through lifestyle changes such as diet, exercise, and stop smoking (Anderson *et al.* 2002; Pirillo *et al.* 2013; Packard *et al.* 2008; Anderson *et al.* 1999; Bazzano *et al.* 2001).

Initiating event in atherosclerosis is the change LDL into oxidized LDL (Ox-LDL) by several factors such as radicals, lypooxygenation, causing splitting of fatty acids that are not saturated into the LDL particle. Ox-LDL activity will be mediated by different receptors known as scavenger receptors (SR), such as SR-AI/II, SR-BI, CD36, MARCO, marcosialin (CD68) and lectin-like oxidized low-density lipoproteins receptor-1 (LOX-1). Sawamura *et al.*, first to identify LOX-1 as a major receptor for endothelial cells in Ox-LDL (Dunn *et al.* 2008; Kurnaz *et al.* 2011). Mango *et al.*, delivers up to now there are 7 polymorphisms that have been identified in the LOX-1 gene. One of them is 3'UTR188CT (Kurnaz *et al.* 2011; Chen *et al.* 2000; Chen *et al.* 2001; Chen *et al.* 2003; Reiss *et al.* 2009; Mango *et al.* 2003).

Consumption of leguminous plants that have been known to contain lots of protein and water-soluble fiber may reduce the risk of coronary heart disease (Bazzano *et al.* 2011). Beans (*Phaseolus vulgaris* L) are a type of legume that has been known to have a lowering lipid profile and diuretic effect. Mackay, *et al.*, conducted the study with the addition of 80 grams of beans in the daily diet, obtained an increase in HDL cholesterol compared with no beans in the diet (Kabagambe *et al.* 2005; Hansen D, 2005; Winhan *et al.* 2007; Mackay *et al.* 1992).

Based on the above description, research on the effect of extract beans to LDL and Ox-LDL and relation of Ox-LDL on LOX-1 gene polymorphism 3'UTR188CT, as preventive risk of atherosclerosis has not been investigated. Therefore this study will find out whether extract bean has effects on levels of LDL and Ox-LDL and relation of Ox-LDL on LOX-1 gene polymorphism 3'UTR188CT.

2. Material and Methods

This study is true randomized experiment with a pretest - posttest design with control group.

2.1 Subjects

Subjects were paramedics at Putri Hijau Hospital, Medan. 40 participants were recruited with inclusion criteria:

1. Normal subjects both men and women aged 30-50 years. 2. Have a complete personal data such as name, address, age, and phone number, or mobile phones. 3. Willing to participate in the study and signed a consent form after getting a description of the study (informed consent). Exclusion criteria : 1. Suffering hypertension, diabetes mellitus (DM), and impaired renal function 2. Intolerance to nuts. Drop out criteria : 1. If during the period of the study subjects died or refused to continue the research. 2. If during the study subjects did not consume bean extract that has been established or suffering severe illness.

2.2 Treatment

Subjects underwent pre treatment for one week (baseline period, starting from H – 6 to day 0), with dietary habits such as usual and asked not to consume any kind of food that comes from the nuts.

On day 0, patients underwent blood sampling for examination LDL, Ox-LDL levels and LOX-1 gene polymorphism 3'UTR188CT. Subjects get an explanation about the dosage and how to take the extract.

Treatments from day 0, subjects were divided into four groups. The first group (A) consumed beans every day as much as 40 grams per day were made in the capsule, the second group (B) 80 grams, the third group (C) 120 grams, while the fourth group (D) taking placebo. Consumption schedule is while eating. Subjects will also call or send short messages service/SMS via mobile phone to remind drinking extract schedule.

2.3 Blood test

Blood samples (Whole Blood) is taken when the study participants were in fasting 10-12 hours using a 10 cc syringe already containing anticoagulant (heparin) on the vein by a trained nurse. The blood sample is then sent to a laboratory for examination of LDL (Multigent Architect), Ox-LDL levels (Mercodia, 2003) and LOX-1 gene polymorphism 3'UTR188CT (PCR - RFLP method) (Noviyanti *et al.* 2007).

2.4 Statistical analysis

LDL and Ox-LDL level data were normally distributed. To evaluate effect of bean extract to LDL and Ox-LDL level were compared with unpaired t-test. To evaluate difference of Ox-LDL level on LOX-1 gene polymorphism 3'UTR188CT using ANOVA. We considered a value of $p < 0.05$ as significant. Data analyses were performed by using SPSS 16.0.

3. Result

From 40 subjects who were willing to follow the study, there were 5 subjects drop out because not consume capsules as has been suggested. The fifth subject is from Group A 1 subject, 3 subjects in group C and group D 1 subject, bringing the total study participants who completed the study 35 study subjects.

Of the 35 subjects, most subjects were women 27 subjects (77%) and men 8 subjects (23%). The baseline characteristics of the subjects by study group can be seen in Table 1.

Table 1. The baseline characteristics of the subjects by study group

Characteristic	Bean extract 40 g (N=9)	Bean extract 80 g (N=10)	Bean extract 120 g (N=7)	Placebo (N=9)
Sex female – no (%)	7 (78)	8 (80)	6 (86)	6 (67)
Old - years	43 ± 4,6*	38,2 ± 5,3**	44,43 ± 4,6***	38,78 ± 5,2
Blood sugar	87 ± 8,7	87,7 ± 8,2	80 ± 7,6	86,78 ± 7
Ureum	20,89 ± 5,6	20,8 ± 5,6	19,43 ± 2,4	18,22 ± 4,6
Creatinin	0,84 ± 0,26	0,81 ± 0,26	0,76 ± 0,16	0,83 ± 0,18
Hypertension – no (%)	-	-	-	-
Dyslipidaemia – no (%)	6 (67)	5 (50)	4 (57)	3 (33)

* $p < 0.05$ compared to extract 80 g

** $p < 0.05$ compared to extract 40 g and 120 g

*** $p < 0.05$ compared to extract 80 g and placebo

From the above table it can be seen that the only significant difference was age between subjects who consumed the extract 40 g / day to 80 g / day, 80 g / day to 120 g / day and between 120 g / day with placebo.

From 35 subjects examined LOX-1 gene polymorphisms that have gained 3'UTR188CT CC genotype as many as 15 subjects (43%), CT 17 subjects (49%) and TT 3 subjects (8%). Test results of LOX-1 gene polymorphism 3'UTR188CT based study group can be seen in the figure 1 below.

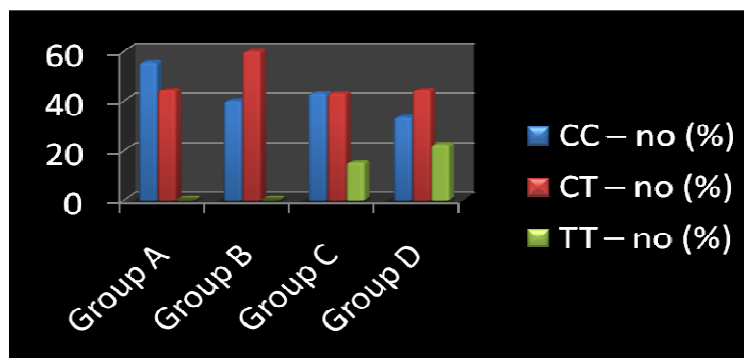


Figure 1. Test results of LOX-1 gene polymorphism 3'UTR188CT based study group
 From 35 subjects who had a C allele were 47 (67%) and T alleles were 23 (33%) that can be seen in the figure 2 below.

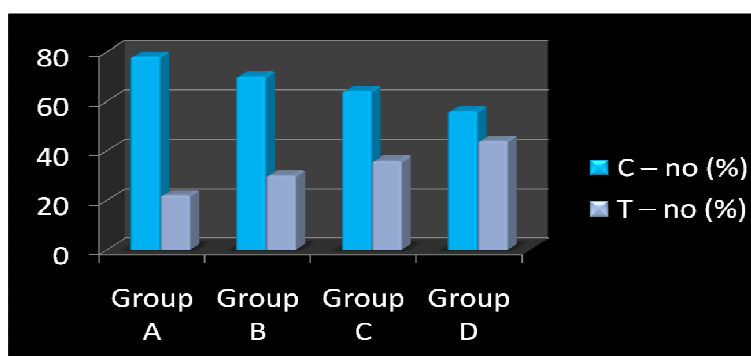


Figure 2. Allele frequency based on study group
 From figure 2, it can be seen that the C allele had a greater frequency than T allele in all groups. TT genotype had higher levels of Ox-LDL plasma than other genotypes both before and after treatment that can be seen in the table below

Table 2. Ox-LDL plasma levels before treatment based on gene polymorphisms LOX-1 3'UTR188CT

Parameter	N	Ox-LDL	p
CC	15	39.9 ± 19.4	0.648
CT	17	42.8 ± 61.2	
TT	3	49.9 ± 8.3	

Table 3. Ox-LDL plasma levels after treatment based on gene polymorphisms LOX-1 3'UTR188CT

Parameter	N	Ox-LDL	p
CC	15	55.93 ± 12.6	0.630
CT	17	53.9 ± 10.3	
TT	3	60.3 ± 5.7	

When compared LDL and Ox-LDL plasma level before and after treatment, the results can be seen in the figure 3 below.

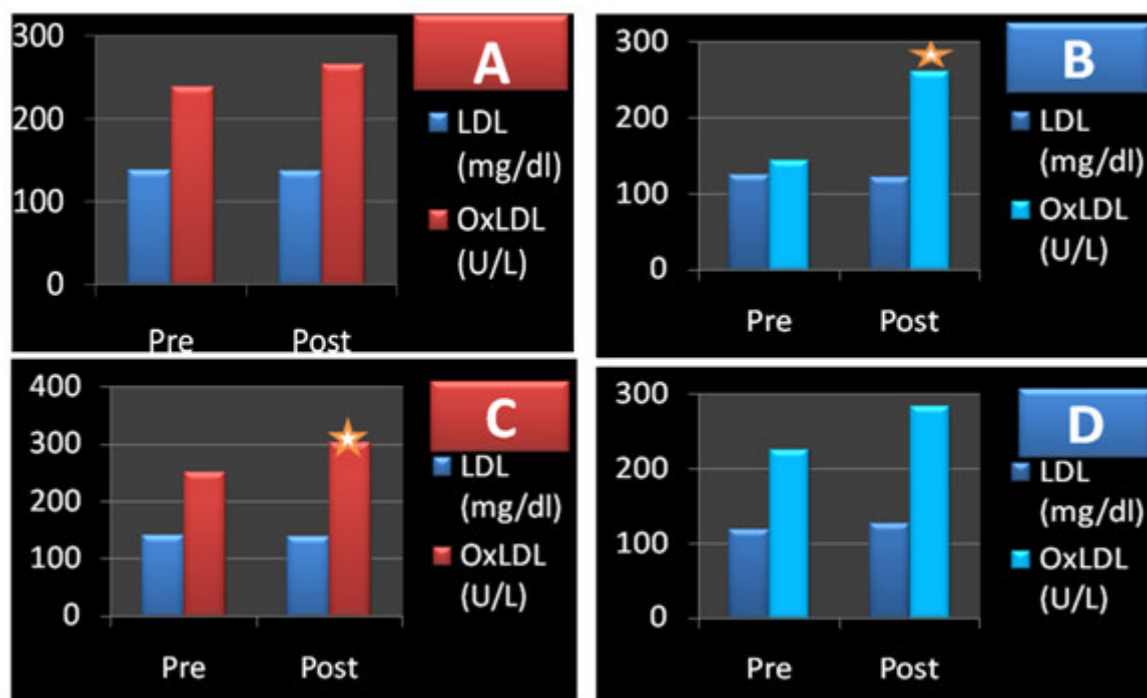


Figure 3. Comparison results of LDL and Ox-LDL plasma level before and after treatment
 From figure 3, it can be seen that the LDL cholesterol decreased in all group gave extract, although not significantly. In group B and group C found increased plasma Ox-LDL were significant after treatment.

4. Discussion

Phytochemical screening results simplisia beans containing the compound identified as alkaloids, saponin, flavonoid, triterpenoid/steroid, and glycoside (data not shown). The phytochemical test results show that beans extract do not contain some crude drug material owned by the bean itself like folate, fibre, arginine, amino acid, asparagines, kholina, tannin, fasin, starch, vitamin A, vitamin C, and minerals (copper, calcium, magnesium, iron, potassium) (Hansen D, 2005).

This study found that plasma levels of Ox-LDL increase after giving bean extract. Possibility of that situation because bean extract not contain complete compound as content materials or substances contained in the beans themselves, such as fibre. Meta-analysis conducted by Brown et al, found that soluble fibre can significantly lower total cholesterol and LDL. Mechanism of fibre can lower cholesterol: the ligament fibres with intra-luminal cholesterol during its formation inhibiting the formation of hepatic fatty acid with the production of short chain fatty acids such as acetate, butyrate, and propionate; alter intestinal motility; slowing the absorption of macronutrients resulting in increased insulin sensitivity and increase the feeling of satiety as a whole will reduce input (Brown *et al.* 1999). In addition to fibre, the substance that also contributes to lowering cholesterol but not present in the extract is folate, and vitamin A (Jeyakumar *et al.* 2007).

Results of this study also found reduction in LDL cholesterol in all groups were given the extract of beans, but also found increase in plasma levels of Ox-LDL. This suggests that although LDL cholesterol can be lowered but not automatically Ox - LDL plasma levels will go down well. This result is also in line with research conducted by Fang *et al* and Silante *et al* (Fang *et al.* 2011; Silaste *et al.* 2004).

Increased plasma levels of Ox - LDL is also known to be temporary. Naruko et al, reported an increase in plasma levels of Ox-LDL in patients with acute myocardial infarction of approximately 3.5 times compared to normal subjects. But the increase in plasma levels of Ox-LDL then down to near normal level when the patient is discharged from the hospital. The same result was obtained by Uno et al, who observed plasma levels of Ox-LDL in patients with cerebral infarction. Ox-LDL plasma levels of patients with cerebral infarction will increase in the days of the first occurrence of the disease and returned to the normal range within 30 days (Itabe *et al.* 2007; Itabe *et al.* 2011; Naruko *et al.* 2006; Uno *et al.* 2003).

Another possibility to elevated levels of Ox-LDL after consuming bean extract is an antioxidant found in beans extract does not contain sufficient antioxidant activity. It is also said by Silaste et al, who conducted the study with administration of a diet low in fat, low in vegetables, and low-fat diet, high vegetable found an increased Ox-LDL by 27% and 9% sequentially. Another possibility is that because it is not clear how the oxidation of LDL occurs in vivo, trials in humans may be used the wrong anti-oxidant (Silaste *et al.* 2004).

Results of this study also found that participants with the TT genotype had higher levels of Ox-LDL compared to participants with CC and CT genotypes. As has been mentioned before, that the Ox-LDL levels will be elevated in patients with cardiovascular disorders so that participants with genotype TT has the possibility for the occurrence of cardiovascular disorders higher than the other genotypes. This is in line with the study conducted by Mango *et al*, while found that participants with the CT or TT genotype had a greater risk for the development of acute myocardial infarction (Mango *et al*. 2003).

5. Conclusion

We found that bean extract can lowering LDL but not Ox-LDL. Require further study to other antioxidants may have a beneficial effect by lowering the levels of Ox-LDL.

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