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The Effect of Simplex Nanoparticles of *Vernonia amygdalina* Del. on Lipid Profile in Hyperlipidemic Rats

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Abstract: Hyperlipidemia is one of the major risk factor for *Cardiovascular Diseases* (CVD). *Vernonia amygdalina* of the family Asteraceae contains flavonoid, saponin and tannin has been being used for the treatment of hyperlipidemia thereby reducing the risk of cardiovascular disease. Treatment of hyperlipidemia is time consuming and costly. Treatment using natural product has been used widely and believed to be effective with less side effect compared to modern medication. Application of nanotechnology in medicine increases steadily but none has been reported for *Vernonia amygdalina* simplex. The purpose of this study was to evaluate the effect of simplex nanoparticles of *Vernonia amygdalina* on lipid profile in hyperlipidemic rats. Nanoparticles of *Vernonia amygdalina* simplex was prepared by milling method then the characteristics of simplex nanoparticles was analyzed by using SEM (*Scanning Electron Microscope*) and PSA (*Particle Size Analyzer*). The lipid profile was determined by enzymatic colorimetric method. Data was analyzed by *Anova* with *Post Hoc Tukey*. SEM and PSA analyses showed that the morphology of nanoparticle of *Vernonia amygdalina* simplex is spherical shaped with particle size 600-700 nm. The results showed that simplex nanoparticles of *Vernonia amygdalina* with the doses of 100 mg/kg bw, 150 mg/kg bw and 200 mg/kg bw decrease total cholesterol, triglyceride, LDL-C and increase HDL-C level significantly with negative control ($p < 0.05$).
Key words: Nanoparticles of *Vernonia amygdalina,* lipid profile.

Introduction

Hyperlipidemia is one of the major risk factor for *Cardiovascular Diseases* (CVD). Both genetic disorders and diet enriched with saturated fats and cholesterol, contribute to the elevated lipid levels in our population as well as in many other developed countries around the world[1].

Hyperlipidemia is defined as an elevation of one or more cholesterol, cholesterol esters, phospholipids or triglycerides[2]. Treatment of hyperlipidemia is time consuming and costly. Besides, drug used for the therapy of hyperlipidemia like HMG CoA inhibitor have potential for adverse effects such as myositis and myalgia, myopathy and rhabdomyolysis[3]. Treatment using natural product has been used widely and believed to be effective with less side effect compared to modern medication. Phytochemical screening of *Vernonia amygdalina* revealed the presence of of alkaloids, carbohydrates, tannins, saponins and flavonoids[4].

Vernonia amygdalina is a shrub in the family of Asteraceae. The leaves are green with characteristic odour and a bitter and sweet taste[5]. Scientific studies have also reported/confirmed it's anticancer[6], analgesic

and antiplasmodial activities[7], antihyperglycemic[8] and antihyperlipidemic[9,10]. The effects of methanolic extract of *Vernonia amygdalina* (VA) at 200 mg/kg was effective in reducing the levels of plasma and PMF total cholesterol as well as low-density lipoprotein cholesterol in rats fed an high cholesterol diet[9]. The research of Oboh and Enobhayisobo, 2009 showed that administration of an aqueous *Vernonia amygdalina* leaf extract to the hyperlipidaemic rabbits caused a decrease in plasma TC, LDL-C, TAG, and VLDL and an increase in plasma HDL-C concentration[10].

Nanoparticles are the preparations having size in nanometers[11]. Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000 nm. The major goals in designing nanoparticles are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen[12]. The purpose of this study was to evaluate the effect of simplex nanoparticles *Vernonia amygdalina* on lipid profile in hyperlipidemic rats.

Experimental

Plant material:

Simplex Nanoparticles of *Vernonia amygdalina* were obtained from LIPI (The Indonesian Institute of Sciences, Bogor). The leaves of *Vernonia amygdalina* were obtained from botanical garden at Faculty of Pharmacy in North Sumatera University, Indonesia and were identified at the *Herbarium Bogoriense* in Botany department, Research Center for Biology in The Indonesian Institute of Sciences, Bogor.

Chemicals:

All reagent kits used for enzyme assay were obtained from Dialab. All other reagents used were of analytical grade and were prepared in all glass distilled water. Simvastatin was a production of Kimia Farma. The food of test animal was pellets BR-II.

Nanoparticles of *Vernonia amygdalina* simplex procedure:

Nanoparticles of *Vernonia amygdalina* simplex was prepared by milling method in The Indonesian Institute of Sciences by using HEM (High Energy Milling). Then, the characteristics of simplex nanoparticles was analyzed by using SEM (*Scanning Electron Microscope*) and PSA (*Particle Size Analyzer*).

Simvastatin dose determination:

The dose used for human hyper-cholesterolemia is 10 mg/day. Doses of simvastatin were converted to Rattus norvegicus and it gave result 10 mg/day x 0.018 = 0.18 mg/day/200 g bw.

Preparation of Simplex Nanoparticles *Vernonia amygdalina:*

A total of 100, 150, and 200 mg simplex nanoparticles of *Vernonia amygdalina* was suspended in 10 ml of carboxy methyl cellulose (0.5%).

Animals:

A total of thirty adult male Wistar rats (150-200g) were obtained from Department of Pharmacology, North Sumatera University, (Medan, Indonesia). Animals were acclimatized for one week in the animal house of Department of Pharmacology. The animals were fed with standard pellet diet and water *ad libitum*. The protocol of animal study was approved by the Institutional Animal Ethical Committee.

Induction of experimental hyperlipidemia:

Hyperlipidemic rats were induced with high fat diet (quail egg yolk, repeated heating oil and goat fat at ratio 3:5:2). The high fat diet were given for 30 days, then the rats were divided into 5 groups (n=6) for different treatments. The first group of animals was given standard diet (negative control) (G1). The second group of rats was given simvastatin 0,9 mg/kg BW (G2). The third, fourth and fifth groups of rats were treated with simplex

nanoparticles of *Vernonia amygdalina* 100 mg/kg BW (G3), 150 mg/kg BW (G4) and 200 mg/kg BW (G5). All the treatments were given for 30 days.

Sample preparation for lipid profile test:

Blood was pooled from tail vein after induced with high fat diet and treatment. Serum samples were analyzed for baseline reading to determine total cholesterol, triglycerides, high density lipoprotein (HDL-C) and (LDL-C) low density lipoprotein- cholesterol. Serum triglycerides and total cholesterol, HDL-C were estimated by enzymatic method and using spectrophotometer. LDL-C were estimated indirectly by using formula[13] : LDL-C (mg/dl) = Total Cholesterol – (Triglycerides/5+HDL-C).

Statistical analysis:

The statistical analysis was carried out by one way Analysis of variance and Tukey Post Hoc test. The differences between groups were considered significant at $p < 0.05$.

Result and Discusion

Characterization of Simplex Nanoparticles *Vernonia amygdalina*

Characterization of Simplex Nanoparticles *Vernonia amygdalina* by using Particle Size Analyzer (PSA) and Scanning Electron Microscopy (SEM)**.** Particle size and size distribution are the most important characteristics of nanoparticle systems. They determine the *in vivo* distribution, biological fate, toxicity and the targeting ability of nanoparticle systems. In addition, they can also influence the drug loading, drug release and stability of nanoparticles. Scanning Electron Microscope (SEM) is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern[11]. SEM and PSA analyses showed that the morphology of nanoparticle of *Vernonia amygdalina* simplex is spherical shaped with particle size 600-700 nm (Fig-1).

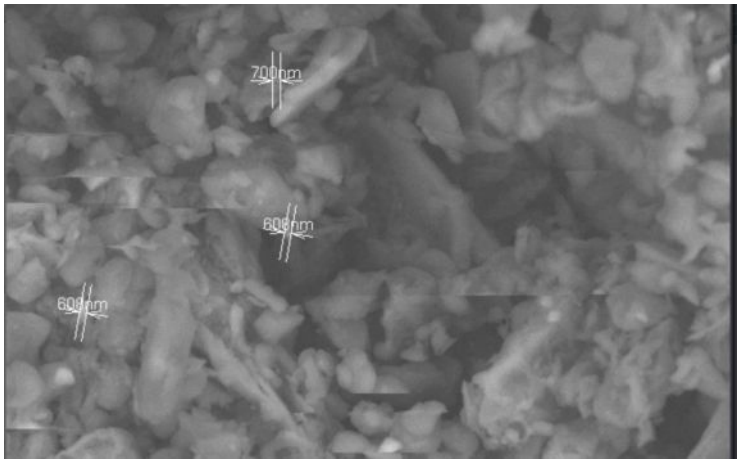

Fig. 1: SEM image of Simplex Nanoparticle *Vernonia amygdalina*

Effect of Simplex Nanoparticles *Vernonia amygdalina* on Lipid Profile (Total Cholesterol, Triglyceride, HDL-C and LDL-C) in Hyperlipidemic Rats

Effect of simplex nanoparticles *Vernonia amygdalina* on total cholesterol in hyperlipidemic rats as shown in Table 1. The result showed that simplex nanoparticles of *Vernonia amygdalina* decreased total cholesterol. The simplex nanoparticles of *Vernonia amygdalina* 100 mg/kg BW are significantly different with simplex nanoparticles of *Vernonia amygdalina* 200 mg/kg BW.

The effect of simplex nanoparticles *Vernonia amygdalina* on triglyceride is as shown in Table 2. The result showed that simplex nanoparticles of *Vernonia amygdalina* 200 mg/kg BW are significantly different with simvastatin in decreasing the triglyceride level.

Effects of the treatments on HDL-cholesterol is shown in Table 3. There are significantly different Simvastatin with simplex nanoparticles of Vernonia *amygdalina* 150 and 200 mg/kgBW in increasing the HDL-cholesterol.

Effect of treatment towards LDL-cholesterol is shown in Table 4. The result showed that there was not significant difference simplex nanoparticles of Vernonia amygdalina with simvastatin in decreasing the LDL-cholesterol.

Hyperlipidemia is caused by a diet high in fat, especially saturated fat and cholesterol[14]. A high fat diet significantly increased the cholesterol total, LDL-cholesterol and triglyceride and decreased the HDL-cholesterol compared to basal diet. Some reports have shown that flavonoids, tannins and saponins may play some roles in antioxidant and hypolipidemic effects[15]. The results of this study showed that the effect of simplex nanoparticles *Vernonia amygdalina* leaves on lipid profil in hyperlipidaemic rats because it contains flavonoid, saponin and tannin present in the simplex nanoparticles *Vernonia amygdalina*.

Table 1 Effect of simplex nanoparticles *Vernonia amygdalina* on total cholesterol in hyperlipidemic rats

Groups	Total Cholaterol average (mg/dl)		
	baseline	induction	treatment
G1	68,50±7,06	145,17±6,36	136,00±2,89
G2	71,00±4,42	145,00±7,38	76,00±4,73*
G3	68,50±5,09	143,33±7,79	79,17±5,03*a
G4	70,17±4,75	146,83±6,73	75,33±6,34*
G5	67,50±5,43	144,83±8,70	70,33±5,81*

Notes: Values as mean ± SD of six animals; *Significantly different from G1 (control negative) group. [a]Significantly different from G5 (simplex nanoparticles of Vernonia amygdalina 200 mg/kg BW) group at $p < 0.05$.

Table 2 Effect of simplex nanoparticles *Vernonia amygdalina* on triglyceride level in hyperlipidemic rats

Groups	(mg/dl)		
	baseline	induction	treatment
G1	73,03±7,50	161,05±9,17	147,10±11,03
G2	83,75±8,17	166,03±6,79	87,07±5,08* [a]
G3	81,60±12,83	168,87±6,20	85,90±5,43*
G4	82,58±10,60	167,75±9,87	79,83±3,83*
G5	83,07±15,41	164,00±10,10	74,85±6,24*

Notes: *Significantly different from G1 (control negative) group, [a]Significantly different from G5 (simplex nanoparticles of *Vernonia amygdalina* 200mg/kg BW) group at $p < 0.05$.

Table 3 Effect of simplex nanoparticles *Vernonia amygdalina* on HDL-cholesterol level in hyperlipidemic rats

Groups	HDL-cholesterol level average (mg/dl)		
	baseline	induction	treatment
G1	35,33±4,41	23,33±2,42	26,50±2,26
G2	36,83±4,26	24,17±2,48	37,67±1,86*[ab]
G3	36,00±4,42	22,50±1,87	41,50±2,42*
G4	35,83±3,06	24,67±1,86	42,83±1,94*
G5	34,67±3,93	23,83±3,06	43,17±2,79*

Notes: *Significantly different from G1 (control negative) group, [a]Significantly different from G4 (simplex nanoparticles of *Vernonia amygdalina* 200mg/kg BW) group. [b]Significantly different from G5 (simplex nanoparticles of *Vernonia amygdalina* 200mg/kg BW) group at $p < 0.05$.

Table 4 Effect of simplex nanoparticles *Vernonia amygdalina* on LDL-cholesterol level in hyperlipidemic rats

Groups	LDL-cholesterol level average (mg/dl)		
	baseline	induction	treatment
G1	18.67±5,24	89,50±5,54	80,00±2,09
G2	17,17±3,87	87,67±7,20	20,83±7,02*
G3	16,17±2,71	87,00±7,56	20,33±4,18*
G4	17,83±3,81	88,50±4,03	16,33±5,46*
G5	16,17±6,04	88,17±7,02	12,17±5,78*

Conclusion

The simplex nanoparticles of *Vernonia amygdalina* with the doses of 100 mg/kg bw, 150 mg/kg bw and 200 mg/kg bw decrease total cholesterol, triglyceride, LDL-C and increase HDL-C level significantly with negative control ($p < 0.05$).

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