

REGULAR ARTICLE

# The effect of crude diosgenin extract from purple and yellow greater yams (*Dioscorea alata*) on the lipid profile of dyslipidemia rats

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## ABSTRACT

The effect of crude diosgenin extracts from purple and yellow greater yams (*Dioscorea alata* L.) tubers on blood lipid profile and fecal cholesterol was studied. Twenty four male Wistar rats were divided into 4 groups: normal group and 3 dyslipidemia groups. Two dyslipidemia groups were administered by crude diosgenin extract from purple and yellow greater yams separately, and one group without administration. The crude diosgenin extracts were orally force-fed at a dose of 88.4 mg/kg body weight for 4 weeks. The results showed that crude diosgenin extract from purple and yellow greater yams improved blood lipid profiles, but did not affect body weight gain. Diosgenin had a role to inhibit cholesterol absorption that indicated by higher fecal cholesterol in treated groups. Crude diosgenin extract from purple greater yam exhibited better lipid profile improvement than crude diosgenin extract from yellow one, indicated by lower total cholesterol, triglyceride, low density lipoprotein (LDL) cholesterol, and higher high density lipoprotein (HDL) cholesterol after 4 week treatment. Crude extract of purple water yam also had better inhibition of cholesterol absorption. In conclusion, both crude diosgenin extracts from greater yam increased fecal cholesterol secretion and improved blood lipid profiles.

**Keywords:** Diosgenin; Dyslipidemia; Fecal cholesterol; Greater yam, Lipid profile

## INTRODUCTION

Coronary heart disease is a leading cause of death in the developed and also developing countries (Lloyd-Jones et al., 2010), that one risk factor of this disease is disordered lipid metabolism or dyslipidemia. Dyslipidemia is a lipoprotein metabolism disorder in which blood serum had high level of low density lipoprotein (LDL) and total cholesterol (Rerkasem et al., 2008).

Many studies have been exploring the role of natural bioactive compounds in improving lipid profile of lipid metabolism disorder. One bioactive compound that occurs naturally in yam (*Dioscorea sp.*) tuber is diosgenin (Sautour et al., 2007) that has a role in decreasing hepatic and plasma triglycerides (Uemura et al., 2011). Diosgenin (3 $\beta$ , 25R-Spirost-5-en-3-ol), a plant sterol, is structurally similar to cholesterol (Korster et al., 2005) (Fig. 1). According to Son et al. (2007), diosgenin (a steroidal saponin of yam) has long been used as a raw material for industrial

production of steroid drugs, and reported to have a hypocholesterolemic effect by suppressing cholesterol absorption and increasing cholesterol secretion. Diosgenin is the main active compound in the family Dioscoreaceae (Cayen and Dvornik, 1979) and is a biologically active phytochemical that is used for the treatment of various diseases such as leukemia, inflammation, hypercholesterolemia, and some cancers (Patel et al., 2012). Raju and Bird (2007) showed the expression of 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase at both mRNA and protein levels was significantly lowered by increasing concentrations of diosgenin. HMG-CoA reductase is the rate-limiting enzyme of the cholesterol biosynthetic pathway. Previously, Cayen and Dvornick (1979) indicated that diosgenin suppresses cholesterol absorption in rats. The study concluded that diosgenin interferes with the absorption of cholesterol of both exogenous and endogenous origin; such interference is accompanied by derepressed, i.e. increased, rates of hepatic and intestinal cholesterol synthesis.

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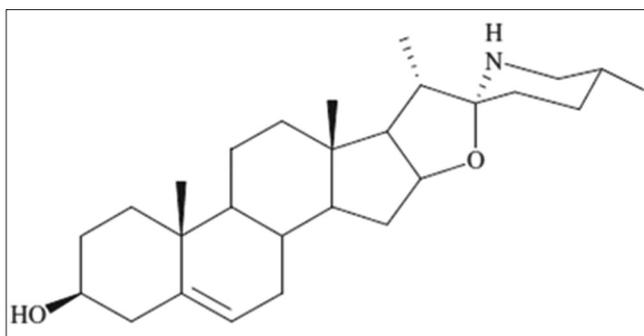


Fig 1. Diosgenin structure (Manrique-Moreno et al. 2014).

One species of yam family is greater or water yam (*Dioscorea alata*). Greater yam tuber contains viscous mucilage which consists of soluble glycoprotein, dietary fiber, and saponin (diosgenin) (Thewles et al., 1993; Jenkins et al., 2001). Greater yam tuber has different flesh color including purple, yellow, and white. Purple and yellow greater yams are reported to contain some bioactive compounds such as water-soluble polysaccharide, dioscorin, and diosgenin (Harijono et al., 2013).

Purple greater yam tuber contains diosgenin 1.55 mg/100 g, meanwhile yellow greater yam tuber contains 0.58 mg/100 g of diosgenin (Harijono et al., 2013). *Dioscorea alata* var. *purpurea* from Mumbai, India contained diosgenin 78 mg/100 g (Shah and Lele, 2012). The difference between purple and yellow greater yam is in nutritional composition and pigment of flesh tubers. Purple greater yam flour contains anthocyanin 2.25-15.3 mg/100 g db depends on purple color intensity of the flesh, meanwhile yellow and orange greater yam flour contains carotene 23.8-132 mg/100 g db (Nadia et al., 2015). Slight difference between purple and yellow greater yam possibly related to the composition of crude extract of diosgenin.

Crude diosgenin extract could be obtained by extraction of the plant material to remove the saponins as such with alcohol, and/or other organic solvents, followed by hydrolysis of the extracted crude saponins with mineral acid then extraction or precipitation of the crude saponins (Hersberg et al., 1956). Purification of diosgenin involved supercritical fluid extraction to obtain crude diosgenin, followed by chromatographic purification. Crude diosgenin extract contained fatty acid as impurities (Xu et al., 2008).

Previous studies examined the effect of diosgenin on reducing blood cholesterol levels by using commercial pure diosgenin (Son et al., 2007; Temel et al., 2009; Gong et al., 2010). Son et al (2007) reported that supplementation of pure diosgenin at level 0.1 and 0.5% into a high cholesterol diet for 6 weeks modulated the lipid profile in the plasma and liver. It is not known the effect of un-purified or crude

diosgenin extract on cholesterol metabolism. This study aimed to investigate the effect of crude diosgenin extract from purple and yellow greater yams on lipid profiles and secretion of fecal cholesterol. It was expected that un-purified diosgenin extract also had beneficial effect on cholesterol metabolism and cholesterol absorption.

## MATERIALS AND METHODS

### Materials

Tubers of two cultivars of purple and yellow greater yams (*Dioscorea alata* L.), obtained from local farmers in Tuban, East Java, Indonesia, were used as raw materials for crude diosgenin extraction.

### Crude diosgenin extract preparation

This method used was according to that described by Trivedi et al. (2007). Thirty grams of greater yam flour were added to a round bottom flask and 100 mL of 2.5 M sulfuric acid in ethanol was added. The mixture was refluxed for 4 h at 80 °C, and subsequently cooled, filtered, and washed with 2.5 M sulfuric acid in ethanol (3×100 mL). The filtrate was diluted twice with water. This solution was extracted with chloroform (4×20 mL) which was then evaporated to obtain the diosgenin in the form of a translucent liquid.

### Diosgenin analysis

Diosgenin analysis was based on the method of Chapagain and Wiesman (2005). Three grams of flour were added to 30 mL of n-hexane, shaken overnight, and then centrifuged at 3500 rpm at 20°C for 18 min. The residue was added by 30 mL methanol, shaken overnight, and then centrifuged at 3500 rpm at 20°C for 18 min to obtain saponin. The supernatant was evaporated with a rotary evaporator. Measurement of diosgenin was done by adding 2 mL of ethyl acetate, 1 mL of reagent A (p-anisaldehyde and ethyl acetate), 1 mL of reagent B (sulphuric acid and ethyl acetate), and then placed in a waterbath at 60°C for 10 min. The mixture was cooled to 25°C for 10 min, subsequently the absorbance was measured at  $\lambda = 430$  nm.

### Animals and experimental groups

Twenty four male Wistar rats (*Rattus norvegicus*) with age of 2-3 months were divided into 4 groups that each comprising 6 animals, namely, negative control (normal rats), positive control (dyslipidemia rats), a dyslipidemia group administered by crude diosgenin extract from purple greater yam, and a dyslipidemia group administered by crude diosgenin extract from yellow greater yam. All groups were fed by a commercial diet *ad libitum*, that comprised of moisture content 12%, protein 15%, crude fat 7%, crude fiber 6%, mineral 7%, calcium 1.1%, phosphor 0.9%, and the remaining was total carbohydrate. This diet was made

from corn meal, soybean cake meal, and fish meal. Before treatment, the rats were adapted for 7 days at 24–28°C room. Dyslipidemia condition was achieved by force feeding 1% pure cholesterol and 0.3% colic acid for one week to obtained total choleterol level > 200 mg/dL. After dyslipidemia condition achieved, crude diosgenin extracts from purple or yellow greater yams were force fed at a dose of 88.4 mg/kg body weight every day. The lipid profile and body weight were measured every week for 4 weeks and fecal cholesterol was measured at the end of experiment.

### Lipid profile analysis

Blood was taken retro orbitally and then centrifuged at 4000 rpm for 15 min at ambient temperature to obtain blood serum. Analysis of serum total cholesterol, LDL cholesterol, and HDL cholesterol was carried out using the CHOD-PAP methods (Cholesterol Oxidase - Phenol Aminophenazone) (Siedel et al., 1983). Meanwhile, triglyceride analysis was conducted using the GPO-PAP methods (Glycerol-3-Phosphate - Phenol Aminophenazone) (Sullivan et al., 1985). Fecal cholesterol concentration was measured by the Liebermann-Burchard method (Plummer, 1977).

### Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by the Tukey HSD test. Values were expressed as the mean ± SD with  $p < 0.05$  considered statistically significant. SPSS version 18.0 was used as the statistical software.

### Ethical clearance

This experiment had been approved for ethical clearance No. 202-KEP-UB 2014 from the Animal Care and Use Committee, Brawijaya University.

## RESULTS AND DISCUSSION

### Diosgenin content of crude extract

Fig. 2 shows that the diosgenin content of crude diosgenin extract from the purple greater yam (31.54 mg/100 g) was higher than that from the yellow one (24.62 mg/100 g). Our previous finding (Harijono et al., 2013) similarly revealed that purple greater yam contained more diosgenin than yellow one. Thus, diosgenin concentration in crude extract from the purple greater yam was higher.

The low level of diosgenin concentration in the crude extract indicated that impurities were accidentally extracted. In the extraction of diosgenin, the first step was hydrolysis with sulfuric acid in ethanol to liberate diosgenin from its binding compounds. Then, diosgenin was extracted by chloroform due to its hydrophobicity. During this step,

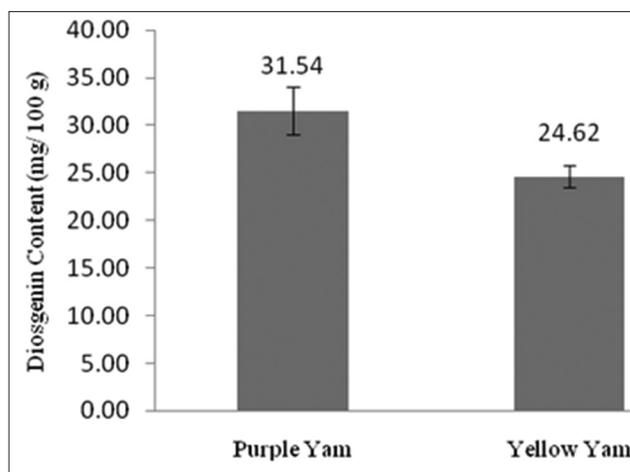


Fig 2. Diosgenin content of crude diosgenin extract from greater yams.

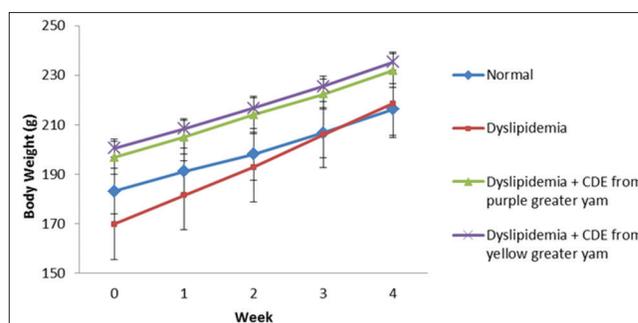


Fig 3. Average body weight gain of group of rats during 4 weeks experiment (CDE = crude diosgenin extract).

other hydrophobic compounds could have been also extracted. Xu et al. (2008) reported that fatty acids were obtained in separation of diosgenin from crude extract of rhizome of *Dioscorea*. Steroids are lipophilic and thereby have the potential to interact with hydrophobic compounds (Manrique-Moreno et al., 2014). Liberation of diosgenin from its binding compounds is difficult because diosgenin binds to sugars (Rahu and Rao, 2012) and can interact with proteins (Tohda et al., 2012; Manivannan et al., 2013). Therefore, impurities in the crude extract of diosgenin may consist of complex compounds.

### Changes in body weight and fecal cholesterol secretion

There was an increase in body weight of rats in all groups from week 0 to week 4 of the experiment. The highest increase of body weight was observed in the dyslipidemia control group without crude diosgenin extract administration (Fig. 3). Lipid accumulated in the bodies of rats fed high cholesterol while slight increase was found in groups administered by crude diosgenin extract. This result was in accordance to that reported by Son et al. (2007) which showed diosgenin intake reduce that rate of body weight enhancement. The study by Uchida et al (1984) showed that no statistically significant

changes on body weight gain after diosgenin intake. Actually, diosgenin did not affect body weight gain as inhibition of cholesterol absorption led to the utilization of absorbed fat and thus preventing fat deposition in the adipose tissue.

Fecal cholesterol secretion of the treated groups was higher than non-treated group (Table 1). The highest cholesterol secretion was found in normal group without crude diosgenin extract treatment. High cholesterol secretion indicates a normal lipid metabolism that body excretes free cholesterol into the feces as a mechanism to eliminate cholesterol body. Induction of cholic acid leads to increase cholesterol absorption (Woollett et al., 2004), thereby dyslipidemia group without crude diosgenin extract administration showed the lowest fecal cholesterol. Crude diosgenin extract treated groups showed more fecal cholesterol that indicated the cholesterol absorption inhibition.

Fecal cholesterol levels were used to estimate the amount of cholesterol absorption inhibition by crude diosgenin extract or secreted into feces from indigenous cholesterol. Crude diosgenin extract from purple greater yam was more effective in increasing fecal cholesterol because of higher diosgenin. Temel et al. (2009) revealed that secretion of neutral sterol into feces, such as cholesterol, increased in rats treated with 1% diosgenin due to impaired cholesterol absorption. In line with the increased in secretion of fecal neutral sterol, the absorption of the cholesterol fraction decreased from 83% to 17% in rats fed by diosgenin compared to rats without diosgenin treatment. Similar finding was reported by Uchida et al. (1984) that diosgenin increased fecal excretion of cholesterol, but decreased fecal excretion of bile acids. Diosgenin is known to increase hepatic cholesterologenesis, accompanied by an increase in the secretion of cholesterol into bile. In turn, this can contribute to increased fecal sterols. Also, diosgenin can suppress the absorption of food cholesterol which will move toward the enterohepatic circulation, resulting in an increase of neutral sterol secretion in the feces (Cayen and Dvornik, 1979).

According to Korters et al. (2005), diosgenin has been shown to stimulate biliary cholesterol secretion in mice.

**Table 1: Average fecal cholesterol levels of rats after crude diosgenin extract treatment**

Groups of rats	Fecal cholesterol (mg/100 g)
Normal group	127.01±10.55 <sup>d</sup>
Dyslipidemia	60.29±7.63 <sup>a</sup>
Dyslipidemia+CDE from purple greater yam	112.59±6.31 <sup>c</sup>
Dyslipidemia+CDE from yellow greater yam	89.52±4.99 <sup>b</sup>

CDE: Crude diosgenin extract; (p<0.05)

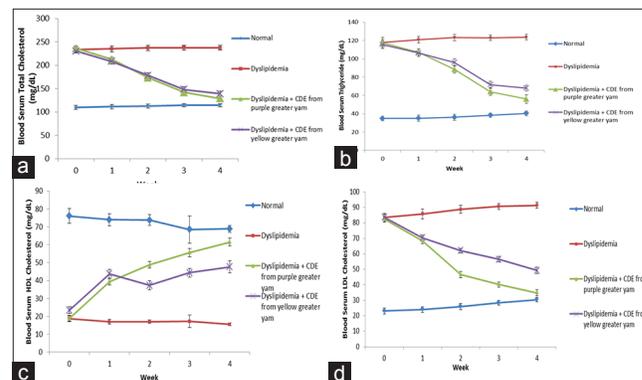
The mechanism of this secretion involved transporter Abcg8 which is essential for most diosgenin-induced biliary cholesterol hypersecretion. Diosgenin probably does not interact directly with Abcg5/Abcg8, but rather increases cholesterol delivery to the heterodimer. Most biliary cholesterol secretion is mediated by Abcg5/Abcg8.

**Blood serum lipid profile**

Total cholesterol level increased 4.7% and 1.6% in normal and non-treated dyslipidemia groups, respectively, during week 4 treatment. Total cholesterol levels in the group administered with crude diosgenin extract from purple and yellow greater yams decreased 45.4% and 39.6%, respectively. The changes of total cholesterol level is shown in Fig. 4a. Crude diosgenin extract from purple greater yam was more effective in lowering total cholesterol compared to the yellow one. It was due to higher diosgenin content in the crude extract from purple greater yam.

Diosgenin is able to decrease serum total cholesterol level by a mechanism similar to cholestyramine (a blood cholesterol lowering drug) (Son et al., 2007). Diosgenin decreased cholesterol by binding bile acids in the digestive tract, interfering with the enterohepatic circulation that made acidic steroid secretion in the feces increase. The decrease in the level of bile acids caused increasing production of bile acids from cholesterol. Enterohepatic circulation was inhibited, therefore the cholesterol absorbed through the gastrointestinal tract was inhibited and secreted into the feces (Malloy and Kane, 2012).

The human liver secretes approximately 1 g of cholesterol per day into the bile. Most of this cholesterol is taken up from the blood in the form of lipoproteins (Kosters et al., 2005). Uptake of blood lipoprotein for hepatic



**Fig 4.** Lipid profile changes of group of rats during 4 week experiment, (a) serum blood total cholesterol, (b) triglyceride, (c) high density lipoprotein (HDL) cholesterol, (d) low density lipoprotein (LDL) cholesterol (CDE = crude diosgenin extract).

cholesterol synthesis contributes to reduction in blood total cholesterol level. In the case of diosgenin, diosgenin decreased cholesterol absorption to about one-third that observed in control mice, an decreased liver cholesterol level (Uchida et al., 1984).

Fig. 4b. shows that, in line with total cholesterol decline, triglyceride level of treated groups also showed significantly reduction by 52.3% and 41.2% for crude extract from purple and yellow water yam, respectively. This result was consistent with that reported by Gong et al. (2010) that diosgenin not only lowered total blood cholesterol level but also lowered blood triglycerides by 35%. Uemura et al. (2011) also showed that diosgenin from fenugreek decreased hepatic and plasma triglyceride and mRNA expression in lipogenic genes. Their finding suggested that diosgenin ameliorated dyslipidemia by decreasing the hepatic lipid content. Kwon et al. (2005) indicated that diosgenin suppressed the time-dependent increase of blood triglyceride levels, suggesting its inhibitory potential against fat absorption. More pronounced effect of crude diosgenin extract from purple greater yam was due to the higher diosgenin content.

Crude diosgenin extract increased serum HDL-cholesterol levels, but untreated control groups showed a decrease (Fig. 4c). At week 4, pronounced effect on HDL cholesterol level increment was found in the group of rats treated by crude diosgenin extract from purple greater yam ( $p < 0.05$ ). Similar findings were reported by Al-Matubsi et al. (2011) and Salimeh et al. (2011) that dietary diosgenin increased HDL cholesterol. Son et al. (2007) showed that supplementation of 0.5% diosgenin in a high cholesterol diet increased serum HDL cholesterol levels by 1.5-fold compared to controls. The role of HDL cholesterol and LDL cholesterol is contradictory. LDL transports cholesterol from the liver to body tissues, while HDL transports cholesterol from the body tissues to liver, preventing the accumulation of cholesterol (Skeaff and Mann, 2012). The increase of HDL cholesterol after crude diosgenin extract administration was possibly related to cholesterol and fat absorption inhibition. Diosgenin inhibited cholesterol absorption, suppressed its uptake in serum and liver, and prevented its accumulation in the liver (Cayen and Dvornik, 1979).

LDL cholesterol level in the normal and dyslipidemia control groups increased by 31.7% and 9.4%, respectively ( $p < 0.05$ ). However, the decrease in LDL cholesterol in the group fed by crude diosgenin extract from the purple yam was greater than the yellow one ( $p < 0.05$ ) (Fig. 4d). Dietary diosgenin elicited a decrease in the synthesis of LDL cholesterol, in accordance with a decline in total

cholesterol level (Cayen and Dvornik, 1979). A decrease in serum total cholesterol led to a decrease in LDL cholesterol. Inhibitory absorption of cholesterol and fat by diosgenin caused an increase in bile secretion into the gastrointestinal tract. Aguilar-Salinas et al. (2010) stated that high secretion of bile indicated that a high amount of cholesterol has been converted into bile for emulsifying fat, therefore serum total cholesterol level and LDL cholesterol decreased.

Cayen and Dvornik (1979) explained that diosgenin treatment increased hepatic and intestinal cholesterol synthesis as well as the activity of hepatic HMG CoA reductase. This was accompanied by increased biliary concentration of cholesterol, but not of bile acids. Diosgenin had no effect on cholesterol synthesis when added to normal rat liver homogenates. The increased unabsorbed cholesterol together with enhanced secretion of cholesterol into bile resulted in increased excretion of neutral sterols without affecting the biliary and fecal excretion of bile acids.

## CONCLUSION

Crude diosgenin extracts from both purple and yellow greater yams improved blood lipid profile and increased fecal cholesterol level. Crude diosgenin extract from purple greater yam in dyslipidemia rats reduced total cholesterol, triglycerides, LDL cholesterol, and increased HDL cholesterol more pronounced than crude diosgenin extract from yellow one. Lipid profile improvement was related to absorption inhibition of cholesterol thus increased fecal cholesterol concentration. This study showed that crude extract of diosgenin also had good effect on lipid profile improvement of dyslipidemia rats.

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### Authors' contributions

All authors contributed equally in conducting the research and in preparing this manuscript.

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