

Hypocholesterolemic and hypoglycemic effects of autoclaved-cooled water yam (*Dioscorea alata*) on hypercholesterolemia rats

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Abstract

This study was inspired due to the potential use of water yams which have high resistant starch therefore they could be developed as functional food. In Indonesia, water yam is differentiated by its flesh color, such as purple, yellow and white. The modified water yam flour was made by three-cycle autoclaving-cooling process in order to increase the resistance starch content (RS3). The effect of modified water yam diet administration on blood cholesterol, glucose and lipid profile of hypercholesterolemia rats was investigated. The results showed that rats which were fed with modified water yam flour had lower concentration of total cholesterol, LDL cholesterol, triglyceride, and blood glucose than those which were fed with standard diet. The higher reduction of total cholesterol and glucose level (39.8% and 58.1% respectively) was found in the group fed with modified white water yam while than those of other groups with purple and yellow water yam diets. It can be concluded that modified white water yam was proved to pose hypocholesterolemic and hypoglycemic effects and could be developed as functional food. The findings of this research can facilitate the development of functional food for the healthiness of human, and for diabetic and hypercholesterolemia patients.

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Introduction

Water yam (*Dioscorea alata*) is known as Ubi Kelapa or Uwi in Indonesia. Water yam is usually differentiated by its flesh color, such as white, yellow and purple water yam. Water yam had prospect to be developed as resistant starch flour because it had higher starch level (52.25%) and amylose level (13.14%) than other tubers, such as *Canna edulis*, *Amorphophallus sp* and *Dioscorea esculenta* (Richana *et al.*, 2004).

Water yam is usually boiled or steamed and consumed as staple food in village society. Being processed, Resistant Starch type III (retrograded starch) is formed and it has positive effect to human health. The previous study revealed that three cycle autoclaving-cooling treatment of arrowroot starch increased resistant content of 10.91% (Sugiyono *et al.*, 2009). Autoclaving-cooling treatments of water yam resulted in a significant increase in crystallinity of the starches, swelling power, and water absorption capacity (Rosida *et al.*, 2017) Autoclaving-cooling

cycle could produce more retrograded amylose fraction (re-crystallization) and was known as RS3 formation (Mutungi *et al.*, 2009). Retrograded amylose (RS3) had heat-stable properties, very complex and resistant to amylase enzyme.

A high cholesterol level in the blood serum is a factor of degenerative disease incident. Anderson *et al.* (2009) stated that an effort in decreasing blood cholesterol level was to consume high dietary fiber food and resistant starch intensively. So *et al.* (2007) reported that feeding RS3 to experimental rats had significant effect on lipid metabolism.

Hypercholesterolemia is a condition which cholesterol level in blood plasma over its normal limit, that is over than 200 mg/L whereas LDL level is over than 130 mg/dL and HDL level is lower than 40 mg/dL. The high cholesterol level in the blood serum cholesterol was one factor which had the risk of cardiovascular disease (Cheng and Lai, 2000). Cholesterol level in the blood was influenced by many factors, such as the amount and kind of fat intake, dietary fiber intake, exercise, and so on. The

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cholesterol reduction could be done by therapeutic diet. Some of dietary fiber and resistant starch could lower LDL cholesterol level. So that consuming high resistant starch food in sufficient quantity could give healthy effect to human body (Anderson *et al.*, 2009)

The consumption of dietary fiber diet intensively in long period of times could reduce serum glucose level, by slowing down of glucose absorption and insulin response. The soluble fiber retarded glucose diffusion and delayed carbohydrate absorption and digestion. So it had positive effect to lower glucose absorption rate so the body had never suffered hyperglycemic condition.

The aim of this study was to evaluate the hypocholesterolemic and hypoglycemic effects of modified water yam on hypercholesterolemia rats. Presumably, the effect in reducing blood glucose level was due to some polysaccharides in water yam, such as resistant starch and dietary fiber.

Table 1. Resistant Starch and Dietary Fiber content of modified water yam flour.

Water Yam Flour	Dietary Fiber (%)	Resistant Starch (%)
Purple	13.04 ^a	7.55 ^b
Yellow	13.42 ^a	7.14 ^b
White	13.81 ^a	9.04 ^a

Materials and methods

Selected purple, yellow, and white water yam tuber (*Dioscorea alata*) were placed in 500 ml beaker glass and heated 15 minutes (121°C) using an autoclave and cooled in a refrigerator for 24 hours (4°C) which was termed as one cycle. The autoclaving-cooling treatment was repeated up to 3 times prior to peeling, slicing, drying and milling in order to make modified water yam flour. Reagent used in this research was analytical grade, such as DiaSys (Diagnostic System) Cholesterol COD FS, DiaSys Glucose GOD FS, DiaSys HDL precipitate, ethanol 96% (pa).

Methods

Selected 32 male Wistar rats (*Ratus norvegicus* Wistar) 2-3 months of age weighing 150-225 g were used in the experiment. Acclimatization was conducted for three days with standard feed of AIN93 (Reeves *et al.*, 1993). The AIN93 standard diet was consisted of corn starch (670,7 g/kg), casein (140 g/kg), sucrose (100 g/kg), soybean oil (40 mg/kg), AIN mineral mix (35 mg/kg), AIN vitamin mix (10 mg/kg), choline bitartrate (2.5 g/kg), L-cystine (1.8 g/

kg), except carboxymethylcellulose addition (fiber-free diets).

After acclimatization, the rats were fed high cholesterol diets for seven days, i.e standard feed which was supplemented with 1% cholesterol and PTU (prophyl thio uracyl), so that all the rats were suffered hypercholesterolemic (cholesterol total over than 200 mg/dL). The rats were randomly assigned into four groups of eight rats per group. Rats in group 1 served as control and fed standard diet (AIN93), while group 2, 3, and 4 were fed purple, yellow and white modified water yam flour respectively. The treatment diet was made from modified water yam flour which replaces corn starch of standard diet. The diet and water were given ad libitum for four weeks. The measurement was performed on days 0, 7, 14, 21 and 28. Blood were taken from a retro-orbital plexus after fasting for 16 hours and were measured for cholesterol total and HDL by CHOD-PAP method; blood Triglyceride by GPO-PAP method and blood glucose by the GOD/PAP method. LDL was measured by Friedewald formulation as followed:

$$\text{LDL} = \text{cholesterol total} - (\text{HDL} + \text{TG}/5)$$

All data were analyzed by Analysis of Variance (ANOVA) and followed by Duncan Multiple Range Test (DMRT's). The in-vivo study have been approved by animal care and use committee of Brawijaya University and declared in the Ethical Clearance No.956-KEP-UB.

Results and discussion

Resistant starch and dietary fiber content

Resistant starch and dietary fiber content was determined enzymatically using modified method of AOAC method 2002.02 (AOAC, 2002). Modification process by autoclaving-cooling cycles in order to increase RS content was reported by many researchers, such as Zabar *et al.* (2008) and Sugiyono *et al.* (2009). In this research, purple, yellow and white water yam flour had resistant starch content of 7.55%, 7.14% and 9.04% respectively. The previous study revealed that water yam modification by three cycle autoclaving-cooling treatment was able to increase RS and DF content, thus able to decrease blood glucose level in hyperglycemia rats (Rosida *et al.*, 2016).

Cholesterol total

After 28 day intervention, serum cholesterol total of purple, yellow, and white water yam group decreased by 39.44%, 37.33% and 39.87%,

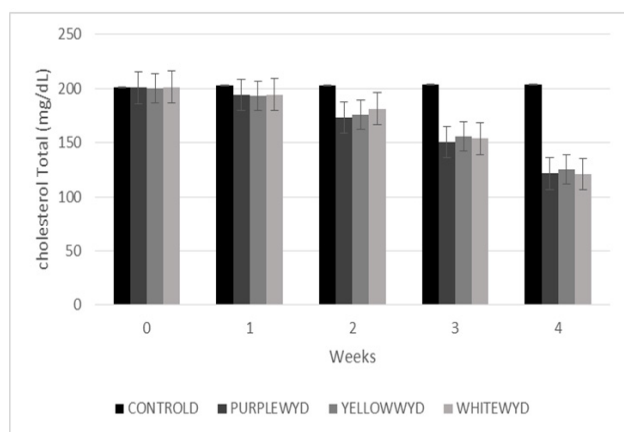


Fig 1. Changes of serum cholesterol total of rats after 28 day intervention by control diet and modified purple, yellow, white water yam diets (WYD).

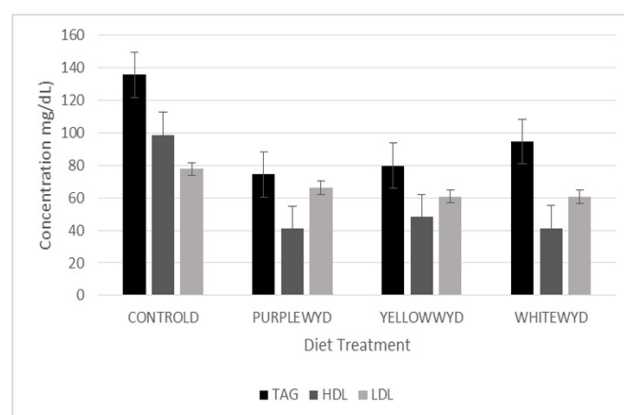


Fig 2. Triglyceride (TAG), HDL and LDL level of hypercholesterolemic rats after 28 days intervention by control diet and modified purple, yellow, white water yam diets (WYD).

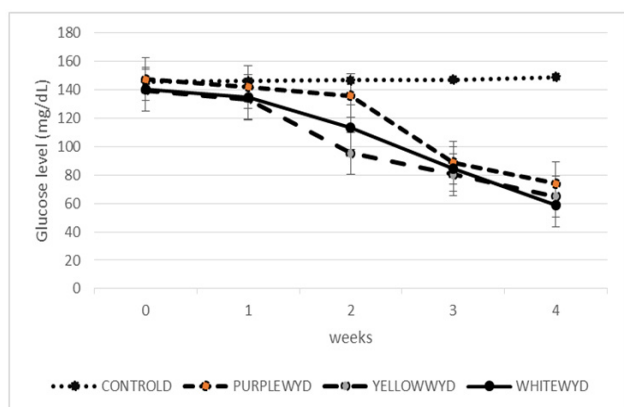


Fig3. Changes of blood glucose level of hypercholesterolemic rats during 4 week feeding of control diet and modified purple, yellow, white water yam diets (WYD).

respectively. However, those of control group were constant (201.5 - 203.8 mg/dL). The data proved that modified water yam flour had potency to reduce blood cholesterol due to its resistant starch and dietary fiber content. So *et al.* (2007) reported that feeding RS3 to experimental rats had significant effect on lipid metabolism. Resistant starch could arrive in

the colon without changing and had the function as dietary fiber.

According to Zhou *et al.* (2015) the lowering of blood cholesterol level in diabetic rats by feeding RS is regulated through promoting lipid oxidation and cholesterol homeostasis even at a moderate level of RS consumption.

Dietary fiber and resistant starch diet could inhibit cholesterol absorption in the small intestine and finally reduce cholesterol level in the blood plasma and increase cholesterol production in the liver, bile acid production and cholesterol excretion in the feces (Anderson *et al.*, 2009).

Triglyceride (TAG), HDL and LDL level

At the beginning, after hypercholesterolemic diet intervention, triglyceride, LDL and HDL level of all rats were not different significantly ($p < 0.05$). However, after feeding treatment, those of modified water yam flour groups were lower than standard diet group. The reduction of cholesterol (Fig1) was in accordance with triglyceride and LDL level reduction (Fig2). It indicated that modified water yam flour had potency in lowering blood cholesterol, LDL and triglyceride level. This phenomenon revealed that modified water yam flour had hypocholesterolemic effect due to its resistant starch and dietary fiber content.

The previous study revealed that experimental rats which were fed modified arrowroot starch had lower LDL cholesterol, triglyceride and total cholesterol, and had higher HDL cholesterol compared to control group (Damat and Haryadi, 2008). According to Zhou *et al.* (2009) total cholesterol and triglyceride level was reduced and HDL cholesterol level was increased in the rats of administration group. The lipid metabolism is modulated through promoting lipid oxidation and cholesterol homeostasis.

The high fiber and high resistant starch diet could increase LDL receptor activity in the liver. This activity fulfilled the availability of tissue cholesterol, so more blood cholesterol was used that reduced blood cholesterol level (Anderson *et al.*, 2009)

Blood glucose level

Statistically, there was a significant decrease ($p < 0.05$) in blood glucose level during 4 week feeding experiment. After 28 day intervention, the serum glucose of purple, yellow and white water yam group reduced by 49.88%, 53.62%, and 58.10%, respectively, while those of standard group were constant (146.01-148.98 mg/dL). The data showed that modified water yam flour was potential in decreasing serum glucose level due to its resistant

starch content. The modified white water yam flour had higher reduction in the cholesterol and glucose level due to its higher RS and Dietary Fiber content than those of purple and yellow ones so that the plan could serve as a great therapeutic diet in the management of diabetes.

The previous study revealed that water yam modification by three cycle autoclaving-cooling treatment was able to increase RS and DF content, thus able to decrease blood glucose level in hyperglycemia rats (Rosida *et al.*, 2016). This study revealed the modified water yam diet can reduce glucose level even at hypercholesterolemia rats.

The availability of resistant starch in small intestine could lower glycemic and insulemic response on diabetic patients (Okoniewska and Witwer, 2007). According to Zhou *et al.* (2015) the lowering of blood glucose level in diabetic rats by feeding RS is regulated through promoting glycogen synthesis and inhibiting gluconeogenesis.

Conclusion

The research revealed that rats which were fed with modified water yam flour had lowered cholesterol total, LDL and glucose and higher HDL level than those of control rats with AIN93 standard diet. The modified white water yam flour had higher reduction in the cholesterol and glucose level due to its high RS and Dietary Fiber content. It can be concluded that modified water yam flour has hypocholesterolemic and hypoglycemic effect on experimental rats.

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