



Bitter Melon (*Momordica charantia* L) Fruit Decreased Blood Glucose Level and Improved Lipid Profile of Streptozotocin Induced Hyperglycemia Rats

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Abstract

Bitter melon (*Momordica charantia*, L) is a fruit that traditionally believe has benefits on health. The objective of this study is to identify bitter melon bioactive and nutritional compounds, and their effect on blood glucose level and lipid profile of streptozotocin induced hyperglycemia rats. Rats were divided into three groups, those were normal group; hyperglycemia group without bitter melon fruit feeding; and hyperglycemia group with bitter melon fruit administration. Hyperglycemia condition was achieved by STZ induction. The experiment was conducted for 4 weeks. The results showed that fresh bitter melon fruit contains β -sitosterol 348.16+1.66 ppm, stigmasterol 183.08+0.8 ppm, campesterol 130.79+0.4 ppm, diosgenin 16.42+0.06 ppm, soluble dietary fiber 2.99+0.07%, insoluble dietary fiber 0.55+0.01%, and pectin 1.41+0.05%. At week 4 of experiment, bitter melon fruit fed hyperglycemia group showed a decrease of 56% blood glucose level compared to blood glucose level at week 0. Body weight of this group also increased. The improvement of lipid profile of bitter melon fed group was indicated by decreasing blood total cholesterol of 49%, triglyceride of 35%, LDL cholesterol of 42%, and increasing HDL of 133% compared to initial level at week 0. Bitter melon also increased fecal cholesterol secretion and effectively inhibited cholesterol absorption in hyperglycemia rats. Bitter melon fruit is suggested for hyperglycemia management due to its ability to reduce glucose and improve lipid profile simultaneously.



Article History

Received: 18 January 2018

Accepted: 23 April 2018


Keywords

Bioactive compounds,
Bitter melon,
Hyperglycemia,
Lipid profile.

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Doi: <http://dx.doi.org/10.12944/CRNFSJ.6.2.11>

Introduction

Momordica charantia, a member of *Cucurbitae* family, is also known as bitter melon, bitter gourd, balsam pear, pare, or karale. Bitter melon is one fruit that usually consumed as vegetable in Asia, India, East Africa, and South America¹. Bitter melon has been believed as one of functional foods that has benefits to reduce blood glucose level² and is used as traditional medicine for the treatment of diabetes³. In spite of its bitter taste, which is unacceptable to some individuals, bitter melon is a very common vegetable and has been consumed in Oriental societies for hundreds of years⁴.

Various studies over the last 20 years have found endocrine and biochemical mechanism behind the hypoglycemic activity of bitter gourd extract². Lucas *et al.*,¹ stated that responsible compounds for many of the proposed health benefits of bitter melon is still in question. Compounds found in bitter melon that possibly contributes to hypoglycemic property are charantin and polypeptide p or plant insulin. The mechanism of action, whether it is via regulation of insulin release or altered glucose metabolism and its insulin-like effect, is still under debate². Bitter melon chemically contains a compound that is very much similar to insulin and sometimes also referred as p-insulin⁵. Furthermore, Lucas *et al.*,¹ explained that tri-terpenoids of bitter melon contributes to improved glucose uptake of cells. Also, protein extract of bitter melon significantly enhanced glucose uptake. Bitter melon also increased hepatic glucose utilization and glycogen synthesis. Another proposed mechanism of action of bitter melon is direct effect on the β cells of pancreas and on the intestinal absorption of dietary glucose. According to Huang *et al.*,⁴ supplementation of bitter melon to high fat diet-fed rats ameliorated the glucose intolerance and hyperinsulinaemia.

However, most of the research on bitter melon mainly focused on antidiabetic properties. In spite the possibility that bitter melon might affect lipid metabolism as well, due to the interconnection between carbohydrate and lipid metabolism⁶. Some studies showed hypolipidemic effect of bitter melon^{4,7}. Senanayake *et al.*,⁶ showed hypolipidemic effect of methanolic extract of bitter melon. This suggested that the methanolic extract of bitter melon contains some components that could ameliorate lipid disorders such as hyperlipidemia.

This fruit contains bioactive compounds such as β -carotene, diosgenin (a steroidal saponin), pectin⁸⁻¹⁰, phytosterols that comprises of stigmasterol, campesterol, and β -sitosterol, also contains soluble and non-soluble dietary fiber¹¹. The oil of bitter melon seed was rich in tocopherols with γ -tocopherol as the major components in all oil samples. Among the phytosterols, sitosterol was the major phytosterol extracted from bitter melon seed oils¹². Kim *et al.*,¹³ also found β sitosterol in bitter melon fruit. Bitter melon fruit is reported to contain steroidal saponin that possesses activities as antidiabetic and antihypercholesterol⁵. Ethanolic extract of bitter melon activated peroxisome proliferator receptors (PPARs), ligand-dependent transcription factors that belong to the steroid hormone nuclear receptor family. This extract also controlled lipid and glucose homeostasis in the body³. Bitter melon also contains charantins abundantly, a mixture of steroidal saponins that have been proposed to have the hypoglycemic and anti-hyperglycemic properties¹⁴.

Hyperlipidemia is one of accompanying side effects of diabetes. Hyperlipidemia in diabetes is also ideally treated by improving glycemic control and lipid profile concomitantly¹⁴. Therefore, it is interesting to find out foods that have glucose and lipid lowering properties simultaneously. We supposed that fresh bitter melon has those properties. This study was objected to evaluate bitter melon fruit in improving blood glucose level and lipid profile of hyperglycemia rats as well as its effect on cholesterol absorption.

Material and Methods

Material

Bitter melon fruits were obtained from local farmers in East Java, Indonesia. AIN 93 M standard diet, streptozotocin (STZ), and nicotinamide (NA) were used as feed and to induce hyperglycemia. Other analytical reagents were used for extraction of bioactive compounds and analysis. DiaSys (Diagnostic System) Glucose GOD FS was used for blood glucose content analysis. DiaSys (Diagnostic System) Total Cholesterol CHOD FS was used for blood cholesterol total content analysis. Total triglyceride BPOP FS, cholesterol LDL CHOD FS, and cholesterol HDL CHOD FS was used for lipid profile analysis.

Bioactive Compound Analysis

Bioactive compounds consisted of diosgenin and phytosterols were determined using LC-MS/MS instrument with MSMS detector (ThermoScientific) type TSQ Quantum Access MAX Triple Stage Quadrupole Mass Spectrometer, UHPLC (ThermoScientific) type Accela 1250, auto sampler (ThermoScientific). Diosgenin extraction procedure used Chapagain and Wiesman¹⁵ method, while the procedure of phytosterols extraction was according to Pereira *et al.*,¹⁶ method. Meanwhile, dietary fiber was analyzed according to method of Asp *et al.*,¹⁷ pectin analysis by method of Egan *et al.*,¹⁸ carotene and beta carotene were referred to method of Horwitz¹⁹.

Diosgenin Analysis

Diosgenin extraction of bitter melon was done using method of Chapagain and Wiesman¹⁵. Dry bitter melon fruit 0.3 – 0.6 g was diluted in 30 ml methanol. The mixture was shake overnight and then centrifuged. The supernatant was collected, and the precipitate was extracted twice using methanol and n-hexane. All supernatant of methanol extract was mixed and the solvent was evaporated using rotary evaporator. The extract was added with 3 ml acetonitrile and filtered with 0.2 µm filter paper. This filtrate was further analyzed by LC-MS/MS. LC-MS/MS operation to test diosgenin uses mobile phase of A=0.1% formic acid in water solution and B = 0.1% formic acid in methanol with isocratic condition. Mobile phase consisted of 70% A and 30% B with 300 µl/min setting speed. LC injection volume was 5µL and the compartment of auto sample (injection temperature) was set 10°C, while column was control (column temperature) at 30°C.

Mass spectroscopy used was MS/MS Triple Q (quadruple) of TSQ QUANTUM ACCESS MAX from Thermo Finnigan with the source of ionization APCI (Atmospheric Pressure Chemical Ionization) was controlled by TSQ Tune software operated with positive mode. The condition of ionization APCI was as follows: the current used was 4µA; 250°C of injection temperature; 300°C capillary column temperature; gas pressure 45 kPa, and Aux gas pressure 15 kPa (arbitrary units).

Phytosterol Analysis

Extraction of phytosterols was performed using method of Pereira *et al.*,¹⁶. As much as 1 g dry bitter melon fruit was saponified with 10 mL of ethanolic 10% KOH solution and sonicated with ultrasound probe for 20 min at 25°C. The mixture was added by 20 mL saturated NaCl solution and 10 mL hexane, and the process was repeated three times. After that, hexane extract was mixed and dried with sodium sulfate anhydrate, and then filtered. After hexane removal, extract was then added by 3 ml acetonitrile. Afterward it was filtered with 0.2 µm filter paper and further analyzed with LC-MS/MS. Column Hypersil Gold (50 mm x 2.1 mm x 1.9 µm) was used in UHPLC (ACEELLA type 1250) from Thermo Scientific. Mobile phase was A = 0.1% formic acid in methanol solution and B = iso-propanol, with the composition of 70% A and 30% B, with 300 µl/min speed. Injection volume was 5µL and injection temperature was 10°C, while column temperature was 30°C.

Blood Glucose, Lipid Profile, and Fecal Cholesterol Analysis

This research had been approved for Ethical Clearance with the approval letter of No. 467-KEP-UB 2015 from Animal Care and Use Committee, Brawijaya University. Male rats (*Rattus norvegicus*) (18 rats, with age 2-3 months) were divided into 3 groups and placed individually in stainless-steel cage. Rats were adapted to laboratory environment for a week. The diabetic condition of rats was achieved by induction with Streptozotocin (STZ) and NA through intraperitoneal injection of 65 mg/kg bw 20. STZ was widely used to induce experimental diabetes in animal by mechanism of action through generating superoxide radicals that damaged β cells of pancreas and led to cell necrosis.

Hyperglycemia was indicated by blood glucose level more 200 mg/dl after 5 d injection. The groups of rats were divided into normal rats with standard diet feeding (negative control group), and hyperglycemia rats with standard diet (positive control), hyperglycemia rats with standard diet and fresh bitter melon fruit feeding. Rats were fed *ad libitum*. Fresh bitter melon fruit juice was force fed to hyperglycemia rats at dose of 71.1 mg/

rat/day. This dose was referred to Gong *et al.*,²¹. During 4-week treatment, blood glucose level, body weight, and feed intake were measured weekly. Blood for analysis of glucose and lipid profile was taken from retro orbital plexus. Blood glucose level was measured by GOD-PAP method (Glucose Oxidize-Phenol Aminophenazone)¹¹. Lipid profile measurement was total cholesterol, high-density lipoprotein cholesterol(HDL-cho), and low density lipoprotein cholesterol (LDL-cho) with CHOD-PAP method (Cholesterol Oxidase-Phenol Aminophenazone)²². Total triglyceride level was measured with GPO-PAP method (Glycerol-3-Phosphate-Phenol Aminophenazone)²³ and fecal cholesterol was measured using Liebermann – Burchard method²⁴.

Analysis of Caecum Digesta Short Chain Fatty Acids (SCFA)

Digesta of caecum was taken after the rats were anesthetized. SCFA was analyzed by gas chromatography from the supernatant of centrifuged digesta (3000 rpm)²⁵. Gas chromatography (Shimadzu GC 2010 plus) was run with injector temperature of 24°C, detector temperature of 240°C, capillary column (RTX-1, 30 m, id. 0.22 mm) temperature of 145°C, and helium as carrier gas (0.96 ml/min).

Statistical Analysis

Nested Design was used as an experimental design. Data was analyzed with one-way analysis of variance (ANOVA) followed by DMRT test (Duncan's Multiple Range Test). Data was presented as mean \pm SD. Data analysis used statistical software SPSS for windows with 18.0 versions.

Results

Bioactive Compounds

Bitter melon fruit contains diosgenin, stigmaterol, β -sitosterol, and campesterol (Table 1). β -sitosterol is the predominant bioactive compounds. Carotene also found in high amount (0.11%), meanwhile fresh bitter melon fruit only contains low level of β carotene. Hyperglycemia rats that fed by bitter melon fruit was received 71.1 mg bitter melon juice per day that contained total carotene 0.078 mg, pectin 1.00 mg, soluble dietary fiber 2.13 mg, insoluble dietary fiber 0.39 mg, diosgenin 0.0012 mg, and phytosterols 0.047 mg.

Table 1: Nutrition and bioactive compounds of fresh bitter melon fruit

Compounds	Content
Water (% , wb)	93.4 + 1.4
Total carotene (% , wb)	0.10+ 0.02
β carotene (ppm, wb)	0.11+ 0.01
Pectin (% , wb)	1.41+0.05
Soluble dietary fiber (% , wb)	2.99 +0.07
Insoluble dietary fiber (%)	0.55+ 0.01
Diosgenin (ppm, wb)	16.42+0.06
β -sitosterol (ppm, wb)	348.16+ 1.66
Stigmaterol (ppm, wb)	183.08+0.8
Campesterol (ppm, wb)	130.79+ 0.4

Body Weight and Blood Glucose Changes

Rat blood glucose level of bitter melon fruit fed group was significantly different ($p < 0.05$) to normal/negative control group and hyperglycemia group without bitter melon fruit feeding positive control group (Table 2). Average body weight of rats fed by bitter melon was higher than that in normal and hyperglycemia control group. It means that bitter melon fruit can improve body weight gain although in hyperglycemia condition. Weekly changes of body weight in four weeks is shown in Fig. 1.

Average increase of body weight in hyperglycemia group administered by bitter melon fruit was 22 g during 4-week experiment, normal rats showed an increase of 35 g, meanwhile hyperglycemia rats without bitter melon fruit feeding showed a decrease of 10 g. It is interesting that bitter melon fruit was able to improve body weight in hyperglycemia condition, and average body weight gain was higher compared to normal rats (Table 2). This finding needs further elaboration in the future work.

Average blood glucose level change during 4-week experiment is shown in Table 2. The lowest blood glucose level is found in bitter melon fruit treated group, although this group was hyperglycemia rats. The average level of blood glucose is lower than normal group (Table 2). Meanwhile, hyperglycemia group that fed only by standard diet still revealed high blood glucose level. Weekly changes of blood glucose level are shown in Fig. 2.

Table 2: Average body weight and blood glucose level after 4-week treatment

Group	Average Body Weight (g)	Blood Glucose Level (mg/dl)*	Glucose Level Change (%)**
Control (-) normal	201.83 + 14.28b	67.80 +2.09a	5.11
Control (+) hyperglycemia	168.60 + 12.74a	220.78+ 6.78c	2.26
Hyperglycemia + bitter melon fruit feeding	206.73+8.94c	51.33+ 4.21b	-56.07

*Different notation in data means the treatment was significantly different at $\alpha=0.05$

**Data in week 4 compared to week 0. Positive (+) means increase, negative (-) means decrease

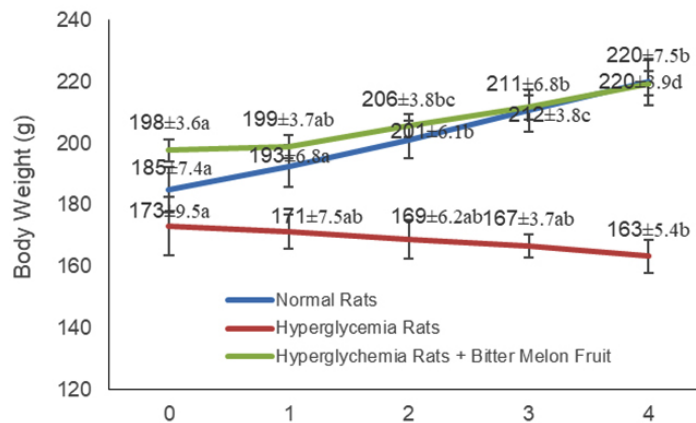


Fig. 1: Body weight changes during 4-week treatment. Data with different notation in the same treatment/group was significantly different at $\alpha=0.05$

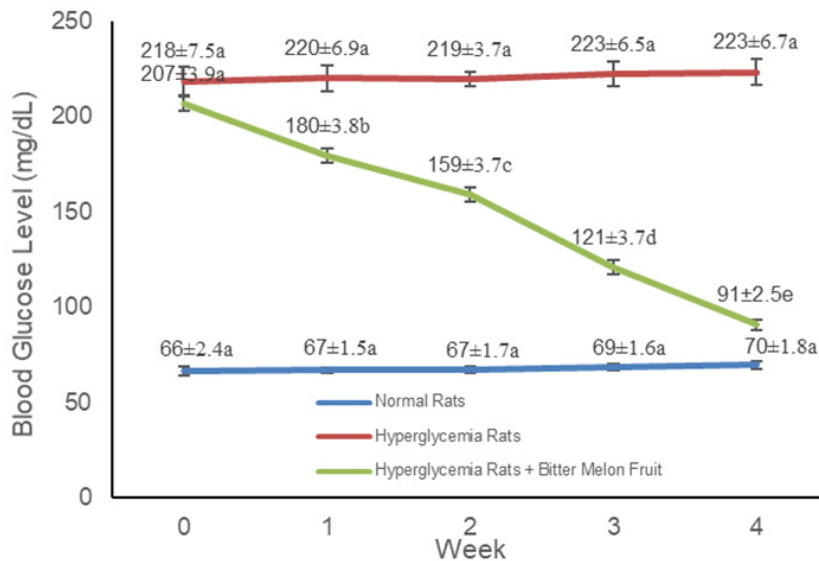


Fig. 2: Blood glucose level changes during 4 week treatment. Data with different notation in the same treatment/group was significantly different at $\alpha=0.05$

SCFAs of *Caecum Digesta*

SCFAs concentration of caecum digesta of bitter melon fruit treated group was higher than control normal and hyperglycemia groups (Fig. 3). The predominant SCFA in all groups was acetic acid,

followed by propionic and butyric acids, respectively. Normal control group showed higher SCFA concentration than hyperglycemia control group. The production of SCFA is related to the presence of dietary fiber in the diet.

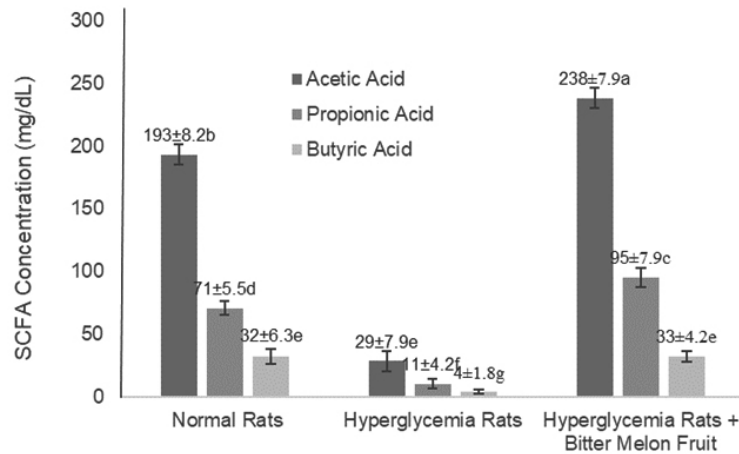


Fig. 3: SCFA of caecum digesta. Data with different notation was significantly different at $\alpha=0.05$

Fecal Cholesterol Level

Fecal cholesterol was analyzed to describe the ability of bioactive and nutrition compounds in bitter melon to inhibit cholesterol absorption. Although cholesterol is also synthesized *de novo*, fecal cholesterol level is an indicator of cholesterol absorption inhibition. The highest fecal cholesterol level is found in normal control group (Fig. 4). Group of hyperglycemia rats treated by bitter melon fruit shows higher fecal cholesterol level than hyperglycemia control group. It means that some compounds in bitter melon fruit are able to inhibit cholesterol absorption. Bitter melon had the ability to increase fecal cholesterol by inhibiting cholesterol absorption. Compared to hyperglycemia rats without bitter melon administration, this hyperglycemia rats showed lower fecal cholesterol level that mean cholesterol was absorbed higher in hyperglycemia condition without bitter melon fruit feeding. Bitter melon had the ability to increase fecal cholesterol although the fecal cholesterol was still lower than normal rats. The increase of fecal cholesterol was 61 mg/100 g due to bitter melon fruit feeding. Without bitter melon feeding, fecal cholesterol was only 41 mg/100 g, and bitter melon fruit administration increased fecal cholesterol to 105 mg/100 g. Compared to normal diet of normal rats, the level of fecal cholesterol of hyperglycemia rats with bitter melon fruit feeding

was lower that meant 4-week feeding of bitter melon fruit was not enough to achieve normal condition. Presumably, longer feeding will result better fecal cholesterol level.

Blood Lipid Profile Improvement

Bitter melon fruit feeding decreased total cholesterol level and LDL cholesterol level of hyperglycemia rat group 97.4% and 51.2%, respectively. Whereas HDL cholesterol level increased 138.5% (Table 3). Hyperglycemia control group exhibited high total cholesterol and LDL cholesterol levels during 4-week experiment, meanwhile normal control group were still able to maintain low total cholesterol and LDL cholesterol levels. Triglyceride of control hyperglycemia rats without bitter melon feeding slightly increased during 4-week experiment. However, hyperglycemia rats fed by bitter melon revealed a decrease in triglyceride.

Before streptozotocin induction, group of rats showed normal lipid profile (Table 3). Group of hyperglycemia rats achieved blood glucose level 5 day after streptozotocin induction and also showed an increase in total cholesterol, LDL cholesterol, and triglyceride, meanwhile HDL cholesterol decreased.

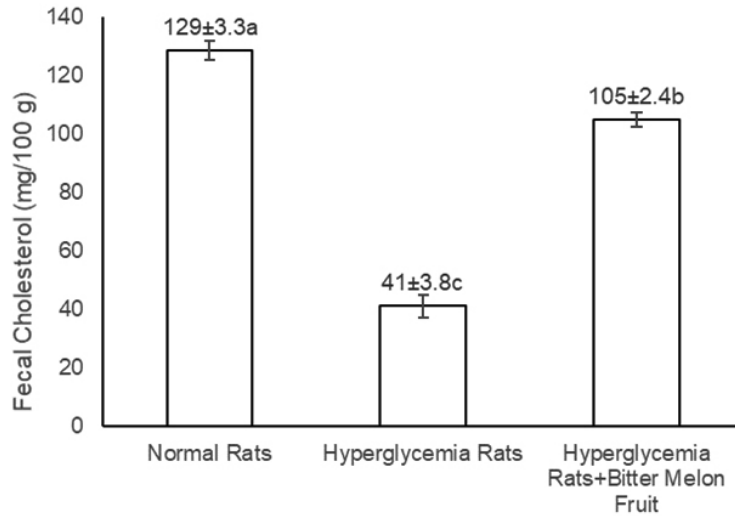


Fig. 4: Fecal cholesterol level. Data with different notation was significantly different at $\alpha=0.05$

Table 3: Lipid profile during 4-week treatment

Group of Rats	Initial*	Week**					Average ***	% Change ****
		0	1	2	3	4		
Total Cholesterol (mg/dL)								
Normal Rats	90±2.1	86.76±2.5a	87.39±2.6a	88.43±2.1a	81.75±2.5b	81.80±2.3b	85±2.4a	-5.72
Hyperglycemia Rats	90±2.5	183.44±4.1a	183.79±4.3a	185.45±4.3a	187.16±3.9a	190.90±5.2a	186±4.4c	4.07
Hyperglycemia Rats + Bitter Melon Fruit Feeding	88±2.1	188.96±2.7a	172.07±3.1b	151.32±2.6c	119.79±2.3d	96.68±3.7e	146±2.9b	-48.84
Triglyceride (mg/dL)								
Normal Rats	78±2.1	74±5.3 a	75±3.6a	76±5.1a	77±2.7a	78±4.5 a	76±4.3a	5.
Hyperglycemia Rats	81±4.0	129±2.7a	129±2.7a	130±2.5a	132±2.6a	132±2.0a	130±2.5c	2.32
Hyperglycemia Rats + Bitter Melon Fruit Feeding	67±2.5	132±5.6a	111±3.5b	102±4.5c	96±2.9c	86±2.7d	106±3.8b	-34.85
LDL Cholesterol (mg/dL)								
Normal Rats	33±1.2	37±3.5a	37±2.1a	38±1.7a	42±1.6a	42±1.7a	39±2.1a	13.51
Hyperglycemia Rats	25±2.2	76±1.4	77±1.2c	79±1.2c	83±1.7c	84±1.4c	80±1.4c	10.53
Hyperglycemia Rats + Bitter Melon Fruit Feeding	30±2.1	71±2.4b	61±2.2b	52±2.5b	41±1.4a	41±1.9a	53±2.1b	-42.25
HDL Cholesterol (mg/dL)								
Normal Rats	54±2.6	61±1.4a	60±1.0 a	60±1.1a	59±1.7a	58±1.9a	60±2.4c	-4.92
Hyperglycemia Rats	54±2.2	26±1.5b	26±1.4b	25±2.1b	24±1.3b	23±2.1b	25±1.6a	-11.54
Hyperglycemia Rats + Bitter Melon Fruit Feeding	63±2.4	24±1.8b	38±1.7c	44±1.0c	51±2.4c	56±2.3a	42±1.9b	133.34

*Before streptozotocin induction

**Data with different notation in the same treatment means significantly different at $\alpha=0.05$

Discussion

Bioactive Compounds of Bitter Melon Fruit

Bitter melon fruit has high total carotene that achieves $0.10 \pm 0.02\%$, but β carotene is only 1.5 ppm. This total carotene content is high compared to other vegetable such as cucumber or broccoli (0.05%)²⁶. The presence of carotene in bitter melon is indicated by the changes of the color of bitter melon fruit from green to yellow during ripening. Zhang *et al.*,²⁷ revealed that concentration of β carotene of unripe bitter melon fruit was higher than ripe fruit.

Total dietary fiber that comprised of soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) of bitter melon fruit is 3.54% (Table 1), this level was similar with that reported by Islam *et al.*,⁷ which found the dietary fiber level of 3.35%. The high dietary fiber content of bitter melon fruit is suitable for dietary fiber supplement for diabetic and pre-diabetic patients²⁸.

One of dietary fiber in bitter melon fruit is pectin which is a soluble dietary fiber that able to retain water and form viscous gel in gastro intestine. Therefore, it slows down the absorption of nutrition including carbohydrate, fat, and cholesterol²⁹. Soluble dietary fiber affects respond of blood glucose in association with the ability to increase food viscosity and, also it is the main factor affecting the rate of glucose absorption. It also increases transition time from gastro intestine and small intestine so that nutrition absorption decreases. This condition makes slow increase in blood glucose level that decreases the requirement of insulin as well.

Bitter melon fruit contains diosgenin, a steroidal sapogenin that also found in other studies. Diosgenin content in this study (Table 2) was lower compared to the study of Mao *et al.*,³⁰ that found diosgenin content of 20,000 ppm. Diosgenin is not only able to bind sugars but also proteins^{31,32}. Diosgenin plays a role to improve profile lipid by suppressing a cholesterol absorption and increase cholesterol secretion through biliary excretion. The changing of biliary cholesterol secretion was associated with expression of hepatic ABCG5 and ABCG8 that are very essential genes involved in cholesterol transport from hepatocytes to bile. Diosgenin decreased the blood plasma total cholesterol and increased HDL

cholesterol levels by mechanism of binding bile salt in gut³³.

Bitter melon has high phytosterols with concentration of 662.03 ppm (Table 1). Previous study showed the concentration of phytosterols on bitter melon seed is 4,705 ppm³⁴. Phytosterols have a role to decrease blood cholesterol level³⁵ by interference in cholesterol synthesis³⁶.

Body Weight Changes

Hyperglycemia induced by STZ was indicated by the severe loss of body weight³⁷. However, group of rats fed by bitter melon in hyperglycemia condition showed an increase in body weight (Fig. 1). In contrary, group of hyperglycemia rats without bitter melon feeding revealed a decrease in body weight. Body weight increase of bitter melon fruit treated group is supposed to relate to improvement of metabolism due to bitter melon fruit consumption. The body weight of hyperglycemia rats was lower than normal rats and bitter melon fruit fed rats. The decrease of body weight in hyperglycemia was related to storage protein utilization due to the carbohydrate unavailability. Storage protein in the muscle is used as the source of energy³⁸. Pedada flour fruit that rich in dietary fiber also increased body weight of hyperglycemia rats³⁹.

Blood Glucose Level Changes

Bitter melon fruit feeding decreased blood glucose level on the hyperglycemia rats group due to effect of glucose metabolism. Bitter melon fruit suppressed the increase of blood glucose was presumably related to the existence of pectin and other dietary fiber (Table 1). Predominant soluble fiber in bitter melon is pectin. Pectin is able to form high viscosity in the digestive tract thus decreases postprandial blood glucose by inhibiting glucose absorption. Gel structure of pectin entrap nutrients absorption⁴⁰.

Dietary fiber in this study increased short chain fatty acids (SCFAs) production. SCFA is able to decrease postprandial glucose by increasing blood free fatty acid level. This free fatty acid can inhibit glucose metabolism through GLUT4 transporter activity inhibition⁴¹. Previous study showed that dried bitter melon fruit powder with 10% dietary fiber improved blood glucose level of STZ induced diabetic rats⁴².

The study of Shetty *et al.*,⁴² used dried bitter melon gourd powder that had higher bioactive compounds compared to this study which used high moisture fresh bitter melon juice. However, blood glucose change of hyperglycemia rats fed by fresh bitter melon fruit in this study was 56%.

Short Chain Fatty Acids of *Caecum* Digesta

Bitter melon fruit feeding to hyperglycemia rats group increased SCFAs concentration (Fig. 3). Soluble fiber fermentation produced SCFAs, with the order of acetate, propionate, and butyrate⁴³. Production of SCFAs in this study had similar order with pedada fruit flour with concentration acetate > propionate > butyrate³⁹.

Type and profile of SCFA produced were varied in association with fiber type in the diet. The SCFAs of caecum digesta were acetic acid, propionic acid, and butyric acid⁴³. Bitter melon fruit feeding led to high concentration of acetate production and lower concentration of butyrate. Acetate and propionate have a role in managing lipid metabolism in the body, while butyrate has a role as fuel for mucosal cells of colon⁴⁴. The high concentration of acetate produced by consuming bitter melon fruit contributed to profile lipid improvement.

SCFAs maintain blood glucose level by increasing insulin release from pancreas and controlling glycogen breakdown in liver. SCFAs also involve in glucose absorption in the intestine and stimulates gene expression of glucose transporters. SCFAs also inhibit liver cholesterol synthesis thus reduce blood cholesterol level⁴⁵. Moreover, propionic acid hampers hepatic cholesterol synthesis. High concentration of propionate and butyrate leads to a decrease of acetate molar ratio. Acetate is a precursor for cholesterol synthesis⁴⁶.

Fecal Cholesterol Level

Fecal cholesterol secretion was one of parameters used to predict cholesterol absorption inhibition or neutral sterol release to fecal by diosgenin⁴⁷ and phytosterols. Fecal cholesterol secretion of the hyperglycemia group with bitter melon fruit feeding was higher than hyperglycemia without bitter melon fruit (Fig. 4). Phytosterols also contribute to slow down cholesterol absorption and increases secretion of fecal cholesterol. Phytosterols have a resemble

chemical structure to cholesterol and have higher affinity to the micelles in cholesterol absorption, thus phytosterols replace cholesterol from micelles⁴⁸. Moreover, free phytosterols increase the cholesterol secretion by inducing higher transporter expression ABCG5/G849.

The high fecal cholesterol secretion indicated releasing free cholesterol from the body in normal lipid metabolism⁵⁰. The increase of fecal cholesterol secretion of bitter melon fruit treated group was also caused by the diosgenin. Diosgenin was able to increase fecal cholesterol secretion by stimulating biliary cholesterol secretion and decreased cholesterol absorption in intestine⁴⁷.

Lipid Profile Improvement

High blood triglyceride level has been identified as one of the main factors to insulin resistance syndrome⁵¹. Insulin resistance was a general characteristic of dyslipidemia atherogenic type of diabetes that indicated by an increase of triglyceride and a decrease of cholesterol HDL level. Table 3 shows that triglyceride was still high during 4-week experiment in hyperglycemia rats without bitter melon fruit feeding, meanwhile normal group maintained low triglyceride level. Group of hyperglycemia rats fed by bitter melon fruit feeding showed a gradual decrease in triglyceride level. The increase of triglyceride in dyslipidemia is important for the risk of cardiovascular disease⁵². High blood triglyceride level had been identified as one of the main causes of insulin resistance syndrome⁵¹. Bitter melon fruit is able to decrease 35% of triglyceride in hyperglycemia group. The decrease of triglyceride is related to soluble dietary fiber, mainly pectin. Soluble dietary fiber reduces fat absorption and binds bile acid that increases its secretion. Increasing bile acid secretion leads to fat absorption disorder thus reduces triglyceride⁵³.

Low cholesterol HDL concentration was also identified as one of the main factors of metabolic syndrome. Low HDL cholesterol level of the subjects of insulin resistance in type 2 diabetic was caused by the increase of apoA-I catabolism. In this study, initial HDL cholesterol level of hyperglycemia rats was low and significantly increased to 133% after bitter melon fruit feeding for 4 weeks (Table 3). The increase of HDL cholesterol level was possibly in relation

to cholesterol inhibition absorption of diosgenin, phytosterols, and dietary fiber of bitter melon fruit. Diosgenin inhibited cholesterol absorption and suppressed its level in serum and liver to prevent cholesterol accumulation in liver⁵⁰. Phytosterols had similar chemical structure with cholesterol and had higher affinity to micelles. It replaces cholesterol from mixed micelles therefore inhibits cholesterol absorption⁴⁸. Moreover, dietary fiber had a possibility to increase viscosity of intestinal digesta, and therefore, could decrease cholesterol absorption. A decrease in cholesterol absorption increases HDL cholesterol.

LDL cholesterol level of hyperglycemia group fed by bitter melon fruit decreased 42% (Table 3). Diosgenin and phytosterols as well as dietary fiber in bitter melon fruit were supposed to decrease cholesterol LDL level. Diosgenin could decrease LDL cholesterol synthesis⁵⁰ and, also stimulates bile cholesterol secretion⁵⁴. Dietary fiber and phytosterols in bitter melon fruit inhibited intestinal cholesterol absorption and fat, that caused the increase of bile acid excretion to intestinal lumen. This led to a decrease in bile acid hepatic level. Thereby, it also increased hepatic degradation of cholesterol to bile acid⁵⁵. Low hepatic cholesterol level was the main cause of compensation of increasing hepatic LDL activity in cell receptor (sensitive cell stimulation).

Total cholesterol level of hyperglycemia rats group reduced 49% (Table 3). The decrease of total cholesterol in bitter melon fruit treated group might be due to inhibition of cholesterol absorption by phytosterols, diosgenin, and dietary fiber.

Conclusion

Fresh bitter melon fruit decreased blood glucose level, improved lipid profile, and increased fecal cholesterol in hyperglycemia rats. The decrease of blood glucose level was caused by dietary fiber of bitter melon, mainly pectin as soluble dietary fiber. SCFA concentration increased significantly with bitter melon fruit feeding. SCFA composition of caecum digesta was acetate > propionate > butyrate. Improvement of lipid profile due to bitter melon feeding was through inhibiting cholesterol absorption by diosgenin, dietary fiber, and phytosterols of bitter melon fruit. Bitter melon fruit is suggested for management of blood glucose and cholesterol in hyperglycemia condition.

Acknowledgement

The authors would like to thank to Directorate General of Research Strengthening and Development, Ministry of Research, Technology, and Higher Education, Republic of Indonesia for supporting this study through Doctoral Dissertation Research Grant (Penelitian Disertasi Doktor) with contract number 9.4.2/UN32.14/LT/2015.

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