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Original Research Article

Inulin from *Dioscorea esculenta* and Metformin in Combination Ameliorates Metabolic Syndrome in Rats by Altering Short-Chain Fatty Acids

Anita S. Soetoko[1]*, Dian Cahyaningrum[2], Fajsa Ardiansyah[2], Dina Fatmawati[3]

[1]*Departement of Anatomy, Faculty of Medicine, Universitas Islam Sultan Agung.*
[2]*Undergraduate student Faculty of Medicine, Universitas Islam Sultan Agung.*
[3]*Departement of Biology, Faculty of Medicine, Universitas Islam Sultan Agung*

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ABSTRACT

Metabolic syndrome (MetS) increases the risk of diabetes and cardiovascular diseases. Several studies have highlighted the role of inulin and metformin in metabolic disorders since insulin resistance and gut microbiota are known to play a role in them. This study was aimed at assessing the effect of the combination of inulin from *Dioscorea esculenta* (DC) and metformin on insulin resistance, lipid profile, and short-chain fatty acids (SCFAs) in the MetS rat model. Twenty-five rats were divided into 5 groups consisting of one untreated group and four treated groups that received a single dose of streptozotocin (STZ) and a high-fat diet (HFD) for 35 days to induce MetS. Starting on day 14, three of the treated groups; I, M, and Com were administered inulin DC (360 mg/day, orally), metformin (100 mg/KgBW, intraperitoneally), and a combination of inulin DC and metformin (inulin DC; 360 mg/day, orally and metformin; 100 mg/KgBW, intraperioteneally), respectively. The biochemical parameters (HOMA-IR, serum insulin, glucose, HbA1C, cholesterol, triglyceride (TG), low-density lipoprotein (LDL) and SCFAs (butyric acid (BA), propionic acid (PA), and acetic acid (AA)) were evaluated. This study found that the combination of inulin DC and metformin decreased HOMA-IR, serum insulin, glucose, HbA1C, cholesterol, TG, LDL and increased butyric acid and propionic acid but not acetic acid in MetS model. In conclusion, the combination of inulin DC and metformin ameliorates insulin resistance and the lipid profile in MetS model rats was associated with the changes in SCFAs.

Keywords: Metabolic syndrome, Inulin *Dioscorea esculenta*, Metformin, Short-chain fatty acids.

Introduction

Metabolic syndrome (MetS) is a group of metabolic disorders characterized by central obesity, hypertension, dyslipidemia, low levels of high-density lipoprotein (HDL), and insulin resistance.[1] It has been reported increases the risk of type 2 diabetes and cardiovascular diseases.[2,3]Patients with MetS have a 2x higher risk of death and a 3x higher risk of having a heart attack and stroke compared to healthy people.[4] A study in Kairo showed that 33.3% of patients with MetS had vessel occulation and more severe coronary artery disease.[5] The global prevalence of MetS has been increasing.[6] According to the International Federation of Diabetes criteria, the global prevalence of MetS is 25%.[2] An unhealthy lifestyle is associated with an increased risk of MetS.[7] A long-term high-fat, low-fiber diet can cause damage to the intestinal barrier, leading to chronic inflammation due to the presence of pathogenic bacteria and their products released into the systemic circulation.[8] Futhermore, insulin resistance has been shown to be the major risk factor of MetS. Insulin has been reported to play an important role in the regulation of glucose, lipid, and energy metabolism in the liver, heart, and gastrointestinal tract.[6] Recent studies showed the association between insulin resistance and gut microbiota imbalance.

*Corresponding author. E mail: anitassoetoko@unissula.ac.id
Tel: +246583584

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Improved insulin resistance is associated with the fermentation of prebiotics into short-chain fatty acids (SCFAs) by gut microbiota.[9]
Inulin, a prebiotic, has gained increasing attention due to its beneficial effects on metabolic disorders.[10] Inulin isolated from *Dioscorea esculenta* (DC) has an antidiabetic effect associated with changes in the number and composition of gut microbiota in rodents.[11]
There is no single drug that has been developed to treat MetS.[7] Metformin has been the most extensively studied agent for its potential as a therapy for MetS. Metformin, a biguanide agent, has been shown to be effective in lowering blood glucose levels, improving endothelial dysfunction, and treating lipid metabolic disorders.[12] In addition, metformin has been shown to maintain the integrity of the intestinal barrier and promote the production of SCFAs by modulating the gut microbiota composition.[13]
The combination of inulin and metformin has been known to improve insulin sensitivity in animal models of polycystic ovarian syndrome (PCOS).[14] However, to the best of our knowledge, little is known about the effect of that combination in MetS. This study aimed to determine the effect of the combination of inulin DC and metformin on insulin resistance and lipid profiles in a rat model of MetS indicated by evaluating levels of HOMA-IR, serum insulin, blood glucose, HbA1C, cholesterol, triglycerides (TG), and low-density lipoprotein (LDL). This study also assessed SCFAs serum including propionic acid (PA), butyric acid (BA), and acetic acid (AA) to determine the association between gut microbiota and MetS and its possible underlying mechanisms.

Materials and Methods

Inulin Preparation
Inulin powder was prepared from *Dioscorea esculenta* (DC) tubers using the drying cabinet method. 500 g DC tubers were sliced and made into porridge with the addition of 1000 mL water (1:2) then heated at a

temperature of 80-90^{0}C in a water bath for 30 minutes. The resulting 1300 mL tuber juice was added with 3900 mL ethanol 90% (1:3) and deposited at a temperature of 4^0 C in the refrigerator for 24 hours, after which the precipitate was centrifuged at 1500 rpm for 15 minutes and dried at 37^0 C for 24 hours. The dried precipitate was grounded and then sieved. The levels of inulin derived from DC were determined using a spectrophotometric method with cysteine-carbazole reagent. Inulin levels were calculated based on the standard curve between the standard concentration of inulin compared to the absorbance of inulin at a wavelength of 560 nm.

Ethics statement

The study protocol was approved by the Medical/Health Research Bioethics Commission, Faculty of Medicine, Sultan Agung Islamic University (712/X/2019/Commission on Bioethics).

Experimental animal

Twenty-five male Wistar rats, aged 3 months, were purchased from Animal Laboratory of Universitas Gadjah Mada. The rats were placed and treated in the Animal Laboratory of Integrated Biomedical Laboratory, Universitas Islam Sultan Agung. They were divided into five groups. The untreated group was fed standard diet. Model group of MetS was administrated with a single dose of STZ (10 mg/kg BW; Sigma, USA) freshly dissolved in a 0.1 mol/L citrate buffer and was injected intraperitoneally and were fed a HFD (25g/rat). Inulin DC group (I) was fed the same diet as MetS group and treated with inulin DC (360 mg/days) orally. Metformin group (M) was fed the same diet as MetS group and treated with metformin (100mg/KgBW) via intraperitoneal injection. Combination group (Com) was fed the same diet as MetS group and treated with inulin DC (360 mg/day) orally and metformin (100mg/KgBW via intraperitoneal injection). The composition of the HFD used was 19.9% animal fat, 50% standard diet, 25% wheat flour, 5% quail egg yolk, 10% NaCl, and water. The total energy was 88.67 Kcal/20g, consisting of 48.44% carbohydrates, 21.49% fat, and 14.05% protein. The metformin used is derived from Glucophage XR. Starting on day 14, the three treated groups were administrated with inulin DC and metformin alone or in combination for 21 days. After days 21, the blood sample from each rats in all groups were collected and proceed to biochemical analysis

Biochemical analysis

In this study, the analysed biochemical parameter included HOMA-IR, serum insulin, blood glucose, HbA1C, cholesterol, TG, LDL. Blood glucose levels were evaluated using the glucoDR blood test. HbA1C, cholesterol, TG, LDL levels using spectrophotometry method. Serum insulin levels were analysed using ELISA method. HOMA-IR was calculated as fasting insulin x fasting glucose/ 22.5

SCFAs Analysis

SCFAs were measured using Gas Chromatography-Mass Spectrometry (GC-MS). The following SCFAs were analyzed: acetic acid (AA), propionic acid (PA), and butyric acid (BA).

Statistical analysis

Results were expressed as mean ± SD. The statistical analysis was performed by one-way ANOVA followed by multiple comparison using Graph pad prism 8. *P* value of < 0.05 was considered statistically significant.

Results and Discussion

MetS has been known to be associated with unhealthy diets. Long-term consumption of HFD causes insulin resistance, resulting in metabolic disorders characterized by hyperglycemia, hypertension, dyslipidemia, elevated inflammatory markers, and epithelial dysfunction.[14] A study using laboratory animals fed HFD for 24 weeks showed that insulin resistance increases total cholesterol, TG, LDL, glucose, and serum insulin levels.[15] Other studies using Sprague-Dawley rats fed a combination of high-fat, high-sucrose and STZ injection caused MetS.[16] STZ is a toxic glucose analogue causing pancreatic cellular damage, leading to insulin production disruptions.[17] In our study, the administration of a single dose of STZ and a HFD diet for 21 days was able to induce experimental MetS in rats indicated by a significantly higher HOMA-IR, serum insulin, glucose, HbA1C, cholesterol, TG, and LDL levels compared with the untreated group (p<0.05) (Figures 1 and 2).

The administration of inulin DC and metformin alone or in combination for 21 days showed a significant decrease in HOMA-IR, serum insulin, glucose, and HbA1C levels as compared to the MetS group (p<0.05). The Com group showed a significant decrease in HOMA-IR, glucose, serum insulin, and HbA1C levels compared to M group (p<0.05). There was also a significant difference in HOMA-IR and glucose levels between Com and I group (p<0.05). No significant difference in serum insulin and HbA1C was found between Com and I group (p= 0.683 and p=0.452, respectively) (Figure 1). This result indicate that combination of inulin and metformin have more effective in improving the insulin resistance than metformin and inulin alone. While the combination of inulin and metformin has the same effectiveness with Inulin alone in lowering serum insulin and HbA1C.

A previous study on animal model of PCOS with the combination of inulin and metformin showed improved insulin resistence followed by decrease in plasma lipopolysaccharide (LPS) and proinflammatory cytokines.[13] Systemic inflammation can cause changes in lipid profile.[18,2] This present study supports the effectiveness of inulin DC and metformin to improve lipid profile. As shown in Figure 2, After administration of inulin DC and metformin alone and in combination for 21 days there was a significant decrease in TG, cholesterol, and LDL levels compared to MetS group (p<0.05). There was a significant decrease in TG, cholesterol and LDL levels in Com group compared to I and M group (p<0.05) (Figure 2). This result indicate that combination of inulin DC and metformin have more effectiveness in improving of lipid profile than administration of inulin and metformin alone.

Recent studies has confirmed the role of imbalance of gut microbiota in systemic inflammation due to plasma LPS.[19,20] The previous studies in animal and human gut microbiota showed an association between gut microbiota imbalance and pathogenesis of MetS.[20] Beneficial gut microbiota such as Bifidobacterium spp can improve glucose tolerance and decrease endotoxemia in animal.[21] A study using MetS animal model showed a low number of Bifidobacterium and a high number of *Lachnospiraceae blautia.*[19] In addition, imbalanced proportion of gut microbiota phylum has been associated with a higher number of Firmicutes (94.6%) compared with that of Bacteriodetes *(3.2%)* in gastrointestinal tract in teenagers.[22]

SCFAs, the product of non-digestible fiber fermentation by gut microbiota, are known to have an important role in maintaining intestinal integrity, increasing glucose and lipid metabolism, and regulating the immune system and inflammatory response.[20] In this study, MetS group showed significantly decreased BA and PA levels (p<0.05) but not AA. The administration of inulin and metformin increased the BA and PA levels and combination of both showed the most effect (Figure 3).

Increased BA level is associated with increased insulin sensitivity. In an experimental animal, oral butyrate supplementation activates G-protein couple receptors (GPCR) in the gastrointestinal tract promoting the production of glucagon-like peptide 1 (GLP-1) capable of increasing insulin secretion and reducing insulin serum levels.[12] Besides BA, PA is also known to have a role as a precursor of intestinal gluconeogenesis and inhibit fat synthesis in the liver. Thus, high concentrations of PA reduce insulin resistance and hepatic steatosis in HFD animal model.[23] In this study, the beneficial effect of the combination of inulin DC and metformin was likely to be associated with altered SCFA especially BA and PA. Interestingly, the administration of inulin DC and metformin in this study did not change serum AA levels. This finding is different from that of previous studies showing increased AA in pre-clinical and clinical trials after the administration of inulin and metformin.[11,24]

Acetic acid derived from microbial fermentation of non-digestable fibers and microbial fermentation of residual peptides and fats. [25] This present study showed lower AA levels in rats admistrated with HFD compared to untreated groups but no statistically difference of AA level among all groups. Previous study showing an increased AA in serum and colon after administration of the HFD. This effect is associated with an increase in the number of Firmicutes and decrease in the number of Bacteroidetes as well as increase in serum insulin.[26] In this study, a

combination of inulin DC and metformin decreased serum insulin levels in the MetS rat model. However, no significant changes in AA level were found.

Inulin and metformin are known to improve metabolism via the role of the gut microbiota. The clinical studies on the combination of inulin and metformin showed a significant correlation between the number of microbiota and the clinical parameters of MetS.[27] A previous study showed that inulin improved glucose and lipid metabolism disorders in ob/ob mice through leptin pathway mediated by Prevotellaceae UCG 001.[28] Another previous study in diabetic rat model reported a suppression of proinflammatory cytokine production upon the administration of inulin attributed to reduced number of Mucispirillum and Ruminiclostridium_6.[24] Similarly, metformin has a suppressive response to proinflammatory cytokine.[29] This mechanism was reported by Esteves at al, showing that metformin was capable of suppressing the expression of 11β-HSD1 in human adipocytes. The increase expression of 11β-HSD1 was also found in MetS.[30] Another study on experimental animals fed HFD showed that metformin increases Lactobacillus and restores the expression of sodium glucose cotransporter-1 (SGLT1) and glucose sensing in the upper small intestine.[31] The limitation of the present study is the inability to provide evidence on gut microbiota profile and elucidate molecular inflammatory response associated with imbalance of gut microbiota. However, altered SCFAs could be responsible for the beneficial effect of the combination of inulin DC and metformin.

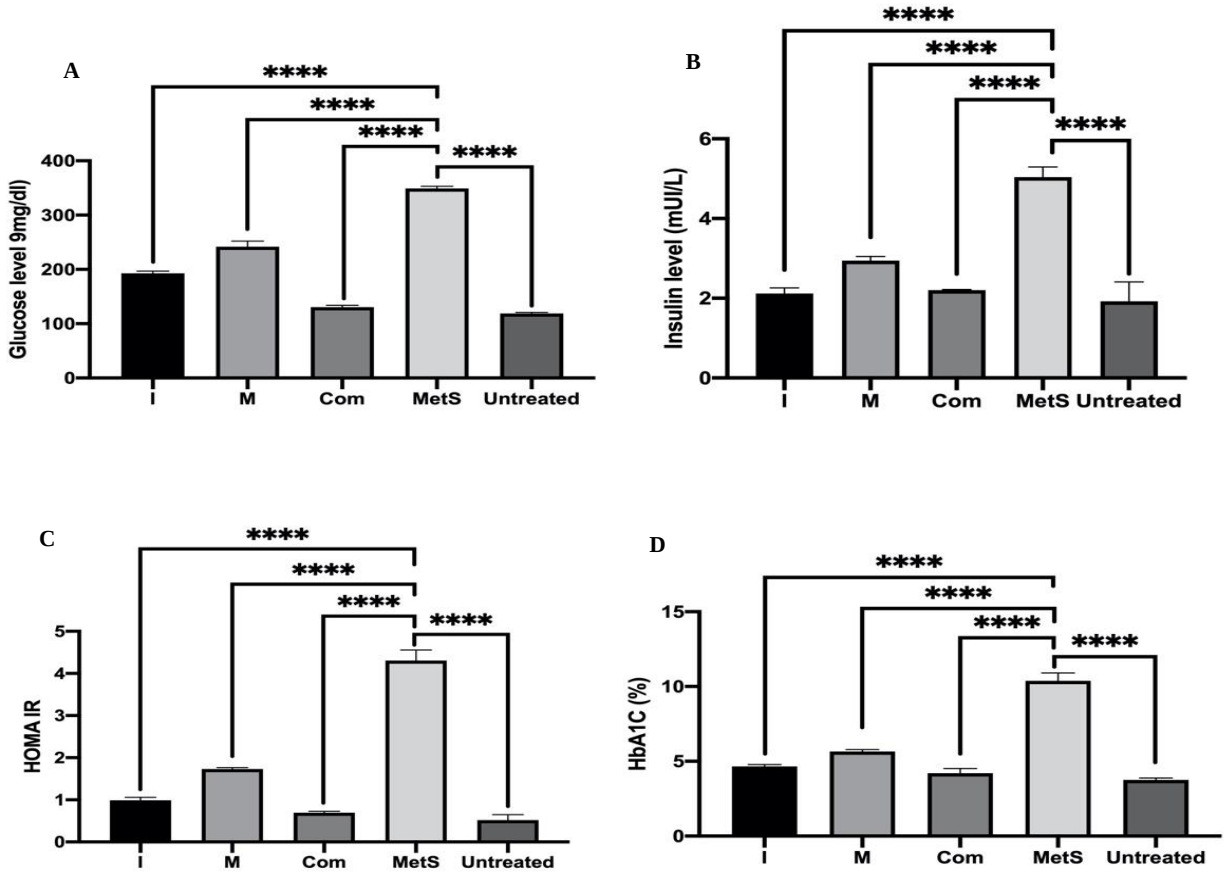

Figure 1: The figure shows the effect of treatment in the study (A) glucose level (mg/dl); (B) Insulin level; (C) HOMA IR; (D) HbA1C (%) in group I (inulin); M (metformin); Com (combination); MetS (metabolic syndrome); untreated. ****Significant difference between groups ($p<0.05$).

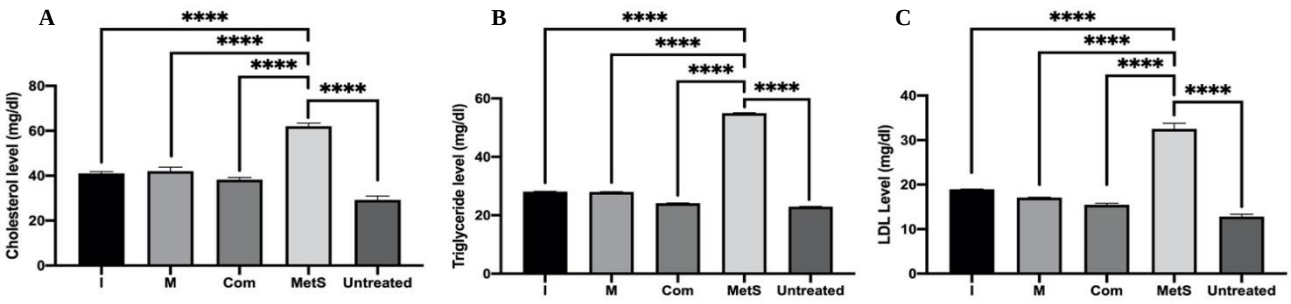

Figure 2: Mean± SD. The figure shows the effect of treatment in the study on several parameters; A. Cholesterol (mg/dl); B. Triglyceride (mg/dl); C. LDL (mg/dl) in group I (STZ+HFD+inulin DC), M (STZ+HFD+metformin), Com (STZ+HFD+combination of inulin DC and metformin), MD (STZ+HFD), untreated group. ****Significant difference between groups ($p<0.05$).

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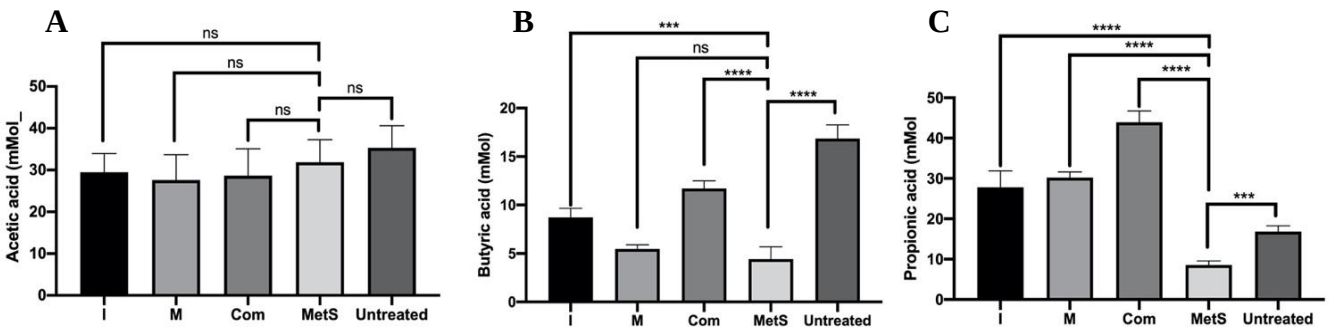

Figure 3: Mean± SD. The figure shows the effect of treatment in the study on several parameters; A. Acetic acid (mMol); B. Butyric acid (mMol); C. Propionic acid (mMol) in group I(STZ+HFD+inulin DC), M (STZ+HFD+metformin), Com (STZ+HFD+ combination of inulin DC and metformin), MetS (STZ+HFD), untreated group. ****Significant difference between groups ($p<0.05$). ns (no significant).

Conclusion

The combination of inulin DC and metformin ameliorates insulin resistance and lipid profile in metabolic syndrome (MetS) model rats associated with the changes in SCFAs. Further studies on the effect of this combination on gut microbiota profile and its molecular response are needed.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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