Dose-dependent Decaffeinated Green Tea Extract Administration Improved Hyperglycemia through Modulation of IRS-1 and GLUT-4 Genes Expression in Metabolic Syndrome Rat Model

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Abstract: Hyperglycemia is a major disorder in metabolic syndrome. Skeletal IRS-1 dan GLUT-4 expression are the key target in hyperglycemia improvement. This study aimed to investigate green tea extract's effect on hyperglycemia improvement in metabolic syndrome rat models. Twenty Sprague Dawley Metabolic Syndrome Rat Model weighed 300 – 400 grams were divided into GTE 200 (n=5) and GTE 400 (n=5) groups. Moreover, as control groups, ten rats were divided into normal control (NC) (n=5) and metabolic syndrome (MS) (n=5) groups. Rats in the GTE 200 and 400 groups were treated once daily with green tea extract at a dose of 200 and 400 mg/bw.t, respectively. The extract was administered for 9 weeks through oral gavage. RT-PCR methods analyzed skeletal IRS-1 and GLUT-4 gene expression. This study showed that the fasting blood glucose of GTE 200 dan GTE 400 was significantly lower than those of the MS group ($p<0.001$ and $p<0.001$, respectively). In addition, GTE 400 group had the lowest fasting blood glucose. Moreover, skeletal IRS-1 dan GLUT-4 gene expression was significantly higher in the GTE 200 and GTE 400 group than those of the MS group. In contrast, the GTE 400 group's gene expression was the highest among all groups ($p<0.000$ and $p<0.002$, respectively). Administration of green tea extract improved hyperglycemia in a metabolic syndrome rat model in a dose-dependent manner through skeletal IRS-1 dan GLUT-4 gene expression modulation.

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1 INTRODUCTION

Metabolic syndrome (MS) is a complex multifactorial disorder that increases the risk of cardiovascular disease and diabetes mellitus type 2. MS prevalence ranges between 10% and 84%, depending on the age, gender, race, ethnicity, and MS criteria.(Alberti et al., 2009) The World Health Organization, International Diabetes Federation, and National Cholesterol of Adult Treatment Panel III (NCEP-ATP III) have determined specific criteria for MS, which includes central obesity, high blood pressure, high triglyceride (TG) levels, low high-density lipoprotein (HDL), and high glucose levels.(Grundy, 2016) There is no single treatment for MS, and natural products have gained attention as potential treatments have increased.(Cerezo et al., 2013; Rohman, 2011)

Tea, the most widely consumed beverage globally, has attracted significant public interest for its potential health benefits.(Cheng et al., 2020; Department of Biology, College of Science, University of Baghdad, Baghdad-Iraq & Al-Hilfy, 2012).

Green tea contains caffeine and polyphenolic compounds, known as catechins. The most abundant catechin found in green tea is (−)-epigallocatechin-3-gallate (EGCG). Tea catechins are also thought to be useful for their antiobesity, antioxidant, antihypertensive, anticarcinogenic, and hypocholesterolemic action. Several studies have described the beneficial effects of tea constituents in animal models of MS.(Ding et al., 2017; Gan et al., 2015; Riegsecker et al., 2013).

Moreover, the skeletal insulin receptor substrate (IRS)/glucose transporter-4 (GLUT-4) pathway has shown to improve hyperglycemia in metabolic disorders in animal models.(Casanova et al., 2019; Cheng et al., 2020) A previous study reported that oral administration of green tea extract (GTE) significantly improved hyperglycemia and increased insulin sensitivity in patients with metabolic disorders.(Wu et al., 2004)

Previous study by Cao et al suggested that green tea extract administration with the dose of 1-2 g/body weight increased IRS1 and GLUT4 mRNA level in skeletal tissue of high fructose diet rats. Moreover, Wu et al suggested that intravenous administration of 0,5 g/100 ml green tea extract alleviated hyperglycemia and increased GLUT mRNA in high fat diet induced rat model for 12 weeks. However, as our knowledge there were limited data regarding the effect of green tea administration on metabolic syndrome rat model.

Therefore, this study aimed to investigate the effect of decaffeinated green tea extract in high fat, high sucrose diet, and low dose streptozococin induced metabolic syndrome rat model for 9 weeks. We hypothesized that decaffeinated GTE modulates IRS/GLUT-4 gene expression and improves hyperglycemia in MS rats.

2 MATERIALS AND METHODS

2.1 Extraction of Green Tea

Green tea was extracted from the young leaves of green tea. Green tea leaves were sorted to obtain high-quality seeds. A dryer cabinet set at 50°C was used to dry 500 g of green tea leaves for 8 h to obtain simplicia with 8%–10% water content. The simplicia was mashed with a blender and macerated with methanol to produce a crude extract. The crude extract was then filtered using a filter cloth to separate the liquid from the solid phase. The liquid phase was concentrated using a rotary evaporator at a temperature of ±40°C. The concentrated liquid phase was partitioned using butanol, water, and acetilacetate Finally, column chromatography was performed using silica gel as the static phase, and the filtered product was evaporated.(Banerjee & Chatterjee, 2014).

2.2 Animal Care and Experimental Protocol

Twenty male Sprague Dawley rats were purchased from the National Agency of Drug and Food Control of Indonesia. They were housed in standard cages and placed in a room where temperature was maintained at 25°C ± 1°C and relative humidity at 50% ± 1%, with a 12 h light/dark cycle. During a 1-week acclimatization period, all rats consumed a normal pellet diet and tap water *ad libitum*. The rats then received a high sucrose, fat, and sodium diet for 9 weeks and an intraperitoneal streptozotocin injection (30 mg/body weight [BW]) in the second and third weeks. Rats with >126 mg/dL blood glucose, >150 mg/dL triglyceride, high systolic blood pressure (≥140 mm/Hg), and reduced HDL levels (<40 mg/dL) were confirmed as MS rats based on NCEP-ATP III criteria.(Saifur Rohman et al., 2017) The rats were divided into four weight-matched groups (n = 5): the normal control (NC), Metabolic syndrome (MS), metabolic syndrome with 200 mg/b.wt GTE (GTE 200), and metabolic syndrome with 400 mg/bw.t GTE (GTE 400). The extract was given via oral gavage

daily. Extract dose was given in milliliters based on the weekly BW measurement. Food and water intake were recorded daily. At the end of the experimental period, animals were anesthetized with ether following a 12 h fasting period. Blood samples were drawn from the heart into a micro-centrifuge tube, and serum samples were obtained by centrifugation at 4000 × g for 15 min at 4°C. The protocol was reviewed and approved by the ethics committee of the Faculty of Medicine, Brawijaya University.

2.3 Physiological Measurement

Daily food and fluid intake were measured on everyday basis, and BW was measured weekly. Food and fluid intake of each rat was measured by subtracting the amount initially provided by the remaining amount in the cage.

2.4 Biochemical Analysis

The serum concentrations of fasting blood glucose, TG, and HDL cholesterol were measured enzymatically using commercial kits (Biolabo, France).

2.5 Blood Pressure Measurements

Blood pressure was measured using the tail-cuff method with a sphygmomanometer at baseline and at the end of the experiment. Three readings were taken consecutively, which were averaged to provide a final systolic Blood Pressure (SBP) reading.

2.6 Gene Expression Analysis

Total RNA was extracted from skeletal tissues with TRIzol Reagent (Invitrogen, USA). The total RNA (2 μg) was reverse transcribed using the SuperScript First-Strand Synthesis System (Invitrogen, USA). Primers were designed according to the sequences in GenBank as follows: β-actin F: "TAC AAC CTC CTT GCA GCT CC," R: "GGA TCT TCA TGA GGT AGT CAG TC;" IRS-1 F: "AAG CAC CTG GTG GCT CTC TA," R: "TCA GGA TAA CCT GCC AGA CC;" and GLUT-4 F: "CTT CCT TCT ATT TGC CGT CCT C," R: "GCT GCT GTT TCC TTC ATC CTG." Standard 25 μL polymerase chain reaction (PCR) with 2 μL of the reverse transcriptase was performed using the following parameters: 95°C, 40 s, annealing temperature, 40 s, 72°C, 45 s, for 27 cycles with TaKaRa Ex Taq Hot Start Version (TaKaRa, Japan) in an MJ Research PTC-200 Peltier Thermal Cycler. The PCR reaction product (10 μL) was separated using 2% agarose gels by electrophoresis. Densitometric quantification of the band intensities was conducted using NIH Image J software.

2.7 Statistical Analysis

All data were analyzed using Statistical Package for Social Sciences (version 22) and are presented as mean ± standard deviation. The data were subjected to a one-way analysis of variance, independent *t*-test, and paired *t*-test and used a significance level of $p < 0.05$.

3 RESULTS

3.1 Baseline Characteristics

Before treatment, the MS rats were characterized by obesity, high systolic blood pressure, high TG, hyperglycemia, and low HDL cholesterol, as shown in Table 1. These characteristics were similar to the MS characteristics observed in humans, according to the NCEP-ATP III criteria.

Table 1. Baseline Characteristics Metabolic Syndrome Rat Model.

Experimental group			
Paramater	NC	MS	p
Body Weight	295,80±5,11	366,2±7,59	0,012
Food Intake	19,40±0,66	22,6±1,67	0,000
Water Intake	26,85±1,92	45,4±14,02	0,001`
Blood Glucose	101,2±4,32	250±40,74	0,000
Triglyceride	81,20±6,76	252,8±66,83	0,000
HDL	44,00±3,46	31,40±6,87	0,000
Blood pressure	124,6±5,5	152,6±7,12	0,000

Values are mean ± SD, n = 5. data was analyzed by dependent t-test.
NC : Normal Control;
MS : Metabolic syndrome Induces

3.2 Effects of GTE on Fasting Blood Glucose Levels

The effect of GTE on fasting blood glucose levels in the experimental animals is presented in Table 2. The level of fasting blood glucose was not significantly

different between any of the groups at baseline. Following 9 weeks of intervention, fasting blood glucose levels in all GTE groups significantly decreased from baseline ($p < 0.001$). Furthermore, all interventional groups showed greater decrease in fasting blood glucose levels compared to that of the MS group ($p < 0.05$). The GTE 400 group had the lowest fasting blood glucose level.

Table 2. The comparison of fasting blood glucose among groups.

Experimental group	Fasting Blood Glucose	
	Pre	Post
NC	101,2±4,32	92,40±9,50
MS	250±40,74	283,2±31,92*
GTE 200	243,89±29,21	196,41±19,19*a
GTE 400	223,40±18,69	181,95±14,95*ab

Values are mean ± SD, n = 5. data was analyzed by dependent t-test.
* : significant between pre test and postest (p < 0,05)
a : significant compared to that MS group (p < 0,05)
b : significant compared to that GTE 200 group (p < 0,05)
NC : Normal Control;
MS : Metabolic syndrome Induces
EGCG 200 : Metabolic syndrome with green tea extract 200 mg/kg.bw.t
EGCG 400 : Metabolic syndrome with green tea extract 400 mg/kg.bw.t

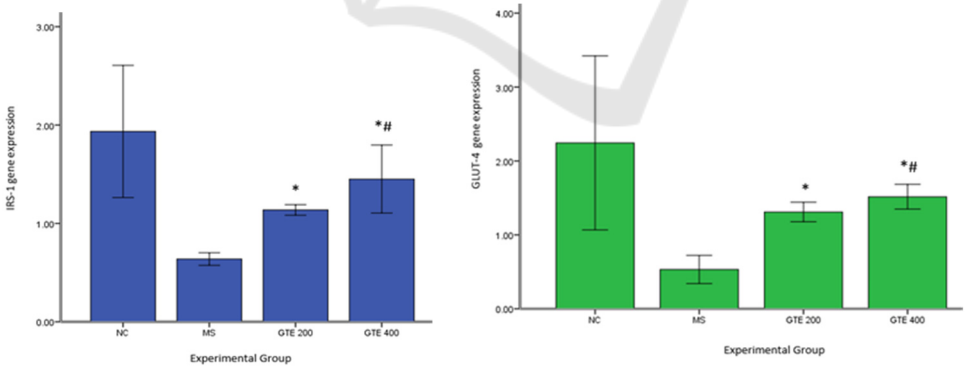
3.3 Effect of GTE on IRS-1 and GLUT-4 Gene Expression

We examined skeletal mRNA gene expression to determine the effect of 9 weeks of decaffeinated GTE administration on IRS-1 (Figure 1a) and GLUT-4 gene expression (Figure 1b). In skeletal tissue, IRS-1 and GLUT-4 gene expression were significantly higher in all GTE groups compared with those of the MS group ($p < 0.001$ and $p < 0.002$, respectively). Moreover, gene expression in the GTE 400 group was the highest among all groups.

4 DISCUSSION

We investigated the effect of decaffeinated GTE on hyperglycemia by modulation of IRS-1 and GLUT-4 gene expression. A recent study was conducted in a rat model that the criteria of MS (hyperglycemia, elevated triglyceride level, decreased HDL level, and hypertension) as presented in baseline characteristics in Table 1.(Saifur Rohman et al., 2017) MS was confirmed by high IRS-1 and GLUT-4 gene expression and significant reduction in fasting blood glucose levels in the GTE 200 and GTE 400 groups. The development of metabolic syndrome rat model in this study was different from previous study that used high fructose diet or high fat diet only or genetically modified rat model. In fact, the rat model in this study represented the features of metabolic syndrome in human, such as hyperglycemia, hypertension, and dyslipidemia.

Hyperglycemia improved after 9 weeks of decaffeinated GTE administration. Furthermore, we



(a) (b)

Figure 1. Effects of green tea extract on IRS-1 (a) and Glut-4 gene expression (b).

Values are mean ± SD, n = 5. data was analyzed by independent t-test.
* : significant compared to that MS group (p< 0,05)
# : significant compared to that GTE 200 group (p< 0,05)
NC : Normal Control;
MS : Metabolic syndrome Induces
EGCG 200 : Metabolic syndrome with green tea extract 200 mg/kg.bw.t
EGCG 400 : Metabolic syndrome with green tea extract 400 mg/kg.bw.t

revealed that one mechanism of hyperglycemia improvement was via the skeletal IRS/GLUT-4 pathway.(Boucher et al., 2014; Chang et al., 2004) Modulation of IRS-1 may have increased GLUT-4 translocation, which induced the reuptake of plasma glucose and improved hyperglycemia. A study by Jang showed that EGCG in green tea reduces fasting glucose and increases insulin and GLUT-4 expression levels in skeletal muscle and adipose tissue.(Fu et al., 2017; Jang et al., 2013) Another study showed that the administration of GTE regulated the expression of genes involved in insulin-signaling pathways in the muscle tissue of rats with MS induced by a high-fructose diet.(Wu et al., 2004) GTE significantly increased mRNA levels of IRS1 and GLUT4 in the muscle tissue. An *in vitro* study by Zhang showed that GTE-rich EGCG improved IRS-1 and GLUT-4 gene expression in L6 muscle cells after dexamethasone induction (Zhang et al., 2010).

A study by Cao showed that GTE at 1 or 2 g/kg BW regulates IRS-1 and GLUT-4 gene expression in rats that are fed a fructose-rich diet. Moreover,(Cao et al., 2007) a study by Cheng et al. showed that administration of 200 mg/b.wt green tea extract decreases fasting glucose, enhances the expression and translocation of GLUT-4, and activates IRS-1 through decreased pSer612IRS-1 expression.(Cheng et al., 2020)

Hyperglycemia alleviation after green tea extract administration might achieved through other pathway such as adiponectin receptor-AMPK pathway, inflammation inhibition pathway by inhibiting gluconeogenesis factor such as FOX-O and PEPCK in hepatic, skeletal, and adipocyte tissue.

Our study showed that GTE 400 had a larger effect on hyperglycemia compared to that of GTE 200, demonstrating a dose effect.(Lukitasari et al., 2018) We used decaffeinated grren tea extract because caffeine may induce palpitations and increase blood homocysteine, which reduced the antioxidant effect of EGCG that was abundantly obtained from tea. Therefore, decaffeinated GTE might minimize these side effects.(Roberts et al., 2015)

5 CONCLUSIONS

Our study revealed the beneficial effect of decaffeinated GTE on hyperglycemia via the modulation of IRS-1 and GLUT-4 receptor gene expression in the MS rat model.

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