

Green Tea Suppresses Serum TNF- α and TGF- β 1 Levels In Mice Model of Systemic Lupus Erythematosus

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Abstract

Objective: This study aims to investigate the effects of green tea administration on TNF- α , Hsp70, and TGF- β 1 levels in the systemic lupus erythematosus (SLE). **Material and methods:** A total of 32 mice will be divided into four groups (each 8 mice), namely the control group, the SLE group, the SLE group who were given green tea extract at a dose of 500 mg/kg body weight, and the SLE group who were given green tea extract at a dose of 1000 mg/kg body weight. Analysis of TNF- α , Hsp70, and TGF- β 1 levels was carried out using the enzyme-linked immunosorbent assay technique. **Results:** TNF- α and TGF- β 1 levels were significantly increased in the SLE group compared to the control group ($p < 0.05$). This increase can be significantly reduced through the provision of green tea, even reaching levels comparable to the control group ($p > 0.05$). **Conclusions:** It was concluded that green tea containing EGCG can suppress TNF- α and TGF- β 1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Keywords: green tea; SLE; inflammation; EGCG; stress protein

Introduction

Systemic lupus erythematosus (SLE) is complex autoimmune and inflammatory disease marked by the presence of autoantibodies against nuclear and cytoplasmic antigens [1]. Worldwide, the average prevalence of SLE is 20-70 per 100.000 individuals annually. The annual incidence of SLE is 10 per 100.000 individuals [2]. The clinical manifestation of this disease varies between individuals. The pathomechanism of SLE is influenced by ethnic, genetic, environmental, and gender factors [3].

Tumor necrosis factor- α (TNF- β) is involved in pathomechanism of SLE, but this detail is still not fully understood [4]. TNF- α levels higher significantly in SLE and higher in active disease status than inactive [5]. TNF- α polymorphism gives sensitivity to individuals and correlates with the disease activity, organ damage, and specific clinical manifestations [6-8]. Increased TNF- α is associated with peripheral blood mononuclear

cell apoptosis [9]. The administration of mesenchymal stem cell infusion can suppress TNF in SLE patients, thereby confirming that this cytokine is the target of SLE therapy [10].

Transforming growth factor- β is a multiregulatory cytokine for the immune system. TGF- β 1 plays a role as regulatory T cell homeostasis in peripheral and T lymphocyte cells [11]. Previous studies have shown a decrease in TGF- β 1 in the plasma of SLE patients, due to the production defect of TGF- β 1 by lymphocytes [12]. This decrease in cytokines parallels the decline in peripheral regulatory T cells [13]. However, other studies have found no changes in TGF- β 1 levels [14,15].

Heat shock protein (Hsp) is a high conserved protein expressed in physiological conditions at low levels. The levels will increase due to the response to stress stimulus [16]. Hsp70 plays a role in inhibiting apoptosis due to stress through various mechanisms. Hsp70 prevents the release of Bax, which is necessary for the

release of proapoptotic factors from mitochondria [17]. Some biofactors were involving in Hsp70 stimulation, including temperature change, inflammatory cytokines, oxidized LDL, and hypoxia. Previous studies have found an increase in CD70 expression in the serum of SLE patients [18].

Tea is a beverage that is consumed by all citizens of the world. This drink is made from a tea plant (*Camelia sinensis* L.). Various evidence states that consumption of green tea is beneficial for health. The biological function of green tea is played by catechin. Of all catechins, EGCG is the most abundant and most functional component [19]. To the best of ours, until now, there have been few studies that have applied EGCG from green tea to SLE. Therefore, this study aimed to know the pharmacological action of EGCG for TNF- α , Hsp70, and TGF- β 1 level modulation in the SLE

Materials and Methods

Animals

Female Balb/c mice, aged 26-28 weeks, weighing 25-30 grams, were randomly divided into four groups (n = 8 each): the control group, the SLE group, the SLE group who received green tea extract at a dose of 500 mg/kilogram body weight (SLE + GT1), and the SLE group who were treated with green tea extract at a dose of 1000 mg/kilogram body weight (SLE + GT2).

Animals were placed in a clean wire cage and maintained according standard laboratory conditions (a temperature of $25 \pm 3^{\circ}\text{C}$ and dark/light cycle 12/12 h). Standard diet and drinking water were provided *ad libitum*. Animals were acclimatized to laboratory conditions for one week prior to the experiment

Pristane-induced SLE

The systemic lupus erythematosus was made by an injection of 0.5 ml pristane (99% pure; Sigma-Aldrich Co, St Louis, MO, USA) intraperitoneally [20].

Plant material

The raw materials of green tea (GMB-4 tea clones) were obtained from the Tea and Quinine Research Center, Bogor, Wes of Java, Indonesia. EGCG as a standard with (95% pure; Sigma-Aldrich Co, St Louis, MO, USA).

Preparation of extracts for LC-MS/MS analysis

Green tea was cleaned and then cooled to dry (a moisture content of 5%). Dried herbs were cut and ground to make a powder. Three hundred grams of powder were macerated in 96% ethanol until the whole powder was submerged (1500 ml). Maceration was carried out for 6 hours with a shaker speed of 40 RPM. The powder bath was refluxed for 3 hours and filtered. The filtering pulp was refluxed again with 96% ethanol, repeated two times. Ethanol contained in the filtrate was removed by a vacuum evaporator at a temperature of 40°C , so that a thick extract of 96% ethanol was obtained.

Standard and sample preparation

The EGCG standard stock solution is prepared by dissolving 5 mg of EGCG in 5 ml of ethanol. Concentrations of standard working solutions are 900 ng/ml; 1300 ng/ml; 1800 ng/ml and 3600 ng/ml.

The extract sample was weighed as much as 1.3 mg and then dissolved in 10 ml of methanol and sonificated for 15 minutes. Centrifugation was carried out at 4000 RPM for 10 minutes. Finally, the sample was taken 20 mL and added to 1 ml of methanol. Samples were filtered with a 0.2 μm filter membrane and were inserted for LC-MS/MS analysis.

LC-MS/MS analysis

LC-MS/MS analysis was performed using UHPLC (ACCELLA type 1250, Thermo Scientific). This instrument was equipped with a vacuum degasser, quartener pump, and thermostat Autosampler. Separation was performed with Hypersil Gold analytic columns (50 mm x 2.1 mm x 1.9 μm). The column temperature was set at 30°C . The Autosampler compartment was set at 16°C . Elution gradients use 0.1% formic acid in water (mobile phase A) and formic acid in methanol (mobile phase B). The elution gradient program is adjusted so that optimum separation occurs, namely 5% B (0-1 minutes), 50% B (5-6 minutes), and 90% B (7-8 minutes). The system is controlled at a constant solvent speed of 300 $\mu\text{l}/\text{min}$ with an injection volume of 2 L.

The TSQ QUANTUM ACCESS MAX mass spectrometer (Thermo Finnigan) is equipped with an Electrospray Ionization source. The Selected Reaction Monitoring (SRM) was applied to quantification

method. The optimization was done in the evaporating temperature of 250°C; capillary temperature of 300°C; nitrogen gas at a pressure of 40 psi; and argon gas at a pressure of 10 psi. Quantitative EGCG analysis was conducted using LC-Quan (Thermo Scientific) software.

Measurement of serum TNF- α levels

Analysis of serum TNF- α levels was performed using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions in the assay.

Determination of serum Hsp70 levels

Analysis of serum Hsp70 levels was performed using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions on the kit.

Analysis of serum TGF- β levels

Analysis of serum TGF- β levels were performed

using an enzyme-linked immunosorbent assay technique. The analysis procedure is carried out according to detailed instructions on the kit.

Statistical Analysis

Data were figured as mean \pm standard of deviation. One-way analysis of variance (ANOVA) with SPSS 15.0 statistical package for Windows was performed for difference analysis. The probability values of $p < 0.05$ were considered statistically significant and later subjected to post hoc test.

Results and Discussion

The chromatogram of EGCG as standard (A) and green tea extract (B). The average concentration of EGCG at crude extracts was 8.08%. Figure 1 displays TNF- α expression in the control group and treatment group. TNF- α levels were significantly increased in the SLE group compared to the control group ($p < 0.05$). This elevation can be significantly normalized through the provision of green tea, even reaching levels comparable to the control group ($p > 0.05$).

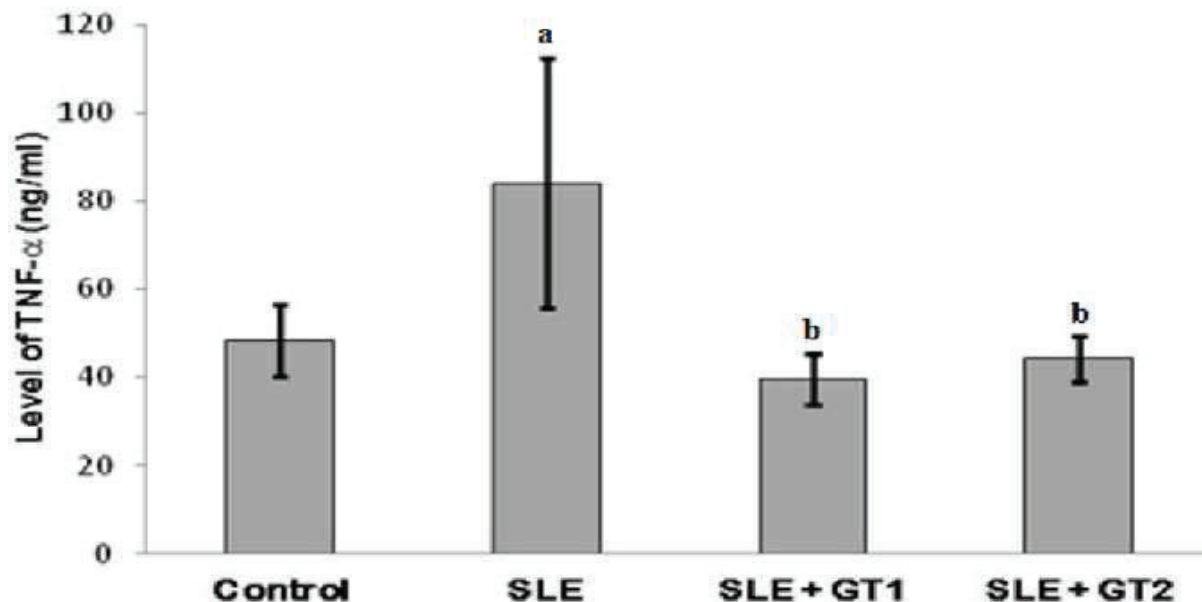
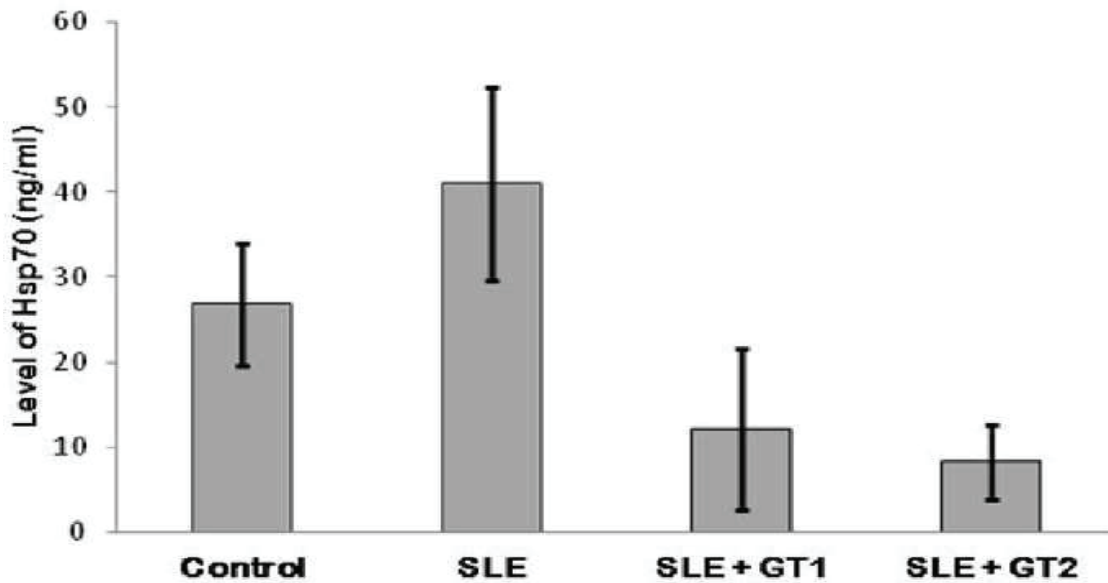


Figure 1. Levels of serum TNF- α in control and other groups. Note: ^a: there are significant differences compared with the control group ($p < 0.05$); ^b: there are significant differences compared with the SLE group ($p < 0.05$); SLE: systemic lupus erythematosus; SLE + GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.



Hsp 70 levels in various groups can be seen in Figure 2. There was an insignificant difference in HSP 70 levels in the control group and the treatment group ($p > 0.05$).

Figure 2. Levels of serum Hsp70 in different groups. Note: SLE: systemic lupus erythematosus; SLE + GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.

Figure 3 shows serum TGF- β 1 levels in various groups. The TGF- β 1 levels was significantly higher in SLE compared to control group ($p > 0.05$). The lowest dose of green tea (SLE + GT1) significantly reduce this levels than that SLE group ($p > 0.05$), reaching comparable value to control group ($p > 0.05$).

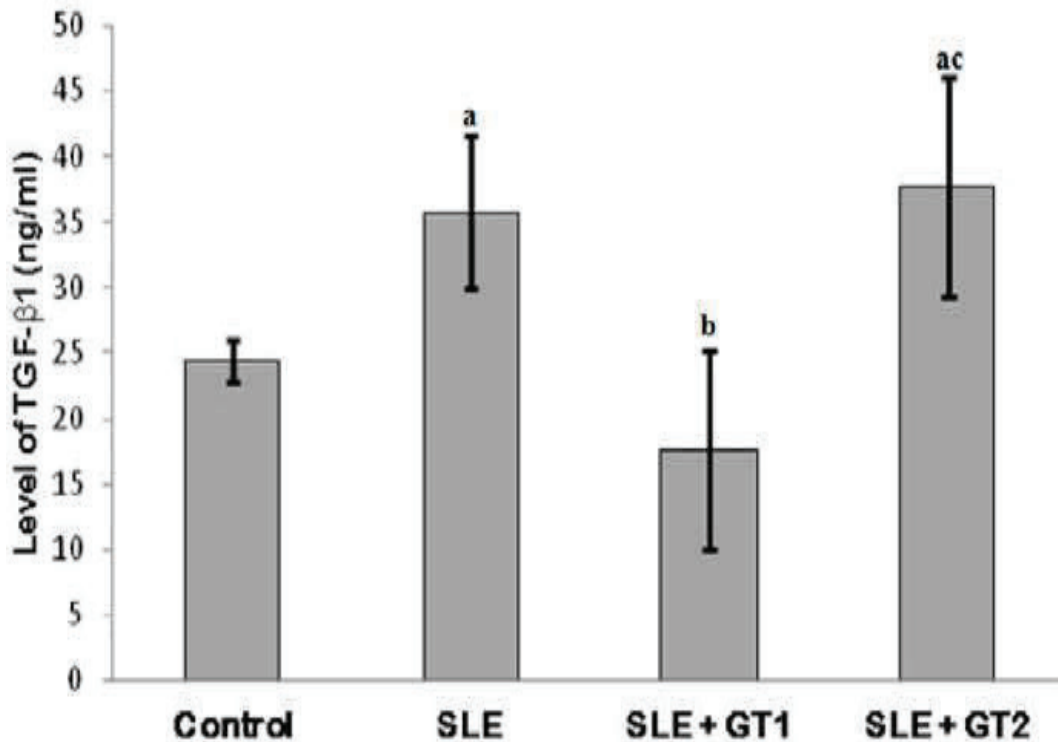


Figure 3. Levels of TGF-b1 in contro and experimental groups. Note: ^a: there are significant differences compared with the control group ($p < 0.05$); ^b: there are significant differences compared with the SLE group ($p < 0.05$); ^c: there are significant differences compared with the SLE + GT1 group ($p < 0.05$); SLE:

systemic lupus erythematosus; SLE

+ GT1: systemic lupus erythematosus treated with green tea at dose of 500 mg/kilogram body weight; SLE + GT2: systemic lupus erythematosus treated with green tea at dose of 1000 mg/kilogram body weight.

Our study focus on the pharmacological effect of green tea in the modulation of TNF- α , Hsp70, and TGF- β 1 in SLE model. We found that the TNF- α levels greater significance in the disease model compared to the control group. This increase indicates the involvement of inflammation in the pathomechanism of SLE. The rise in TNF- α in this SLE model indicated the involvement of kidney manifestations, autoantibodies, and inflammation [21]. Macrophages directly induce TNF- α production in response to immune complexes [22]. Besides, T cells from SLE will activate B cells, display inappropriate homing, and support the creation of proinflammatory cytokines [23,24]. Although this increase is consistent with clinical findings, that TNF- α levels increase in the serum of TNF- α patients [25], some studies state that higher levels of inactive SLE patients indicate that TNF- α can be a beneficial factor in SLE patients [26].

This increase in TNF- α levels in the SLE model of this study could be significantly reduced through the administration of green tea extract, even reaching levels comparable to the control group. This result shows that green tea can downregulate TNF- α production by macrophages in SLE. Our study is confirmed with previous findings that EGCG can suppress inflammation [27]. We hypothesized that green tea and its active compound inhibits transcription factor for TNF- α production. Previous study shows that NF- κ B inducing kinase is a therapeutic target for SLE [28].

For HSP70 levels, there were insignificantly differences between groups. This finding indicates that stress-induced by the administration of the pristane does not trigger Hsp70 production, so that extracellular levels are increased. Extracellular Hsp70 has two roles, namely to induce proinflammatory cytokines and increase antigenicity of antigens through modulation of antigen presentation. Also, extracellular Hsp is anti-inflammatory, triggering negative feedback in controlling inflammation [29,30].

In this study, SLE did not trigger changes in TGF- β 1 levels. This finding is not consistent with previous

clinical studies, that there is no difference in TGF- β 1 levels of SLE patients compared to controls. The second dose of green tea extract can restore TGF- β 1 levels to normal values. The pharmacological action of green tea is supported by previous study [31]. It was concluded that green tea containing EGCG can suppress TNF- α and TGF- β 1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Conclusion

It was concluded that green tea containing EGCG can suppress TNF- α and TGF- β 1 in the SLE model. Thus, green tea can be an alternative in immunology modulation in SLE.

Ethical Clearance-obtained from the ethics commission of Medicine Faculty, Brawijaya University.

Conflict of Interest-no conflict of interests regarding the publication

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