



The effect of green tea extracts on lymphocyte proliferation: A study in mice inoculated with *Listeria monocytogenes*

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KEYWORDS polyphenon-60; Epigallocatechin gallate (EGCG); Epigallocatechin (EGC); lymphocyte proliferation

ABSTRACT Elimination of *L. monocytogenes* in spleen and liver were dependent on CD4 and CD8 T cells. Since previous studies had shown that green tea extract (GTE) stimulated the production of IL-12, IFN- γ , and TNF- α , this recent study was aimed to prove that GTE could induce lymphocyte proliferation. Balb/c mice were randomly divided into 11 groups, each group consisted of 5 mice. Group I, the mice were not given green tea nor bacteria. Group II, the mice were not given tea but they were inoculated with *L. monocytogenes*. Group III to V, the mice were treated with green tea polyphenon-60 in different doses (1.5, 3, and 6 mg/day). Group VI to VIII, the mice were treated with EGCG (0.5, 1, and 2 mg/day). Group IX to XI, the mice were treated with EGC (0.15, 0.3, and 0.6 mg/day). The mice in group III to XI were treated with those GTE orally for 14 days. They were inoculated with 10^4 *L. monocytogenes* intra peritoneally on the 10th day. All the mice were sacrificed on day 15 to examine the number of lymphoblasts in spleens. The results revealed that the number of lymphoblasts increased in all groups of mice treated with green tea polyphenon-60, EGCG, and EGC. Green tea polyphenon-60 had the highest effect to increase the number of lymphoblasts in mice inoculated with *L. monocytogenes*. It was concluded that green tea extracts could induce lymphocyte proliferation.

Listeria monocytogenes could cause a very severe disease with a mean mortality rate in humans of 20 to 30% or higher despite early antibiotic treatment (Schuchat *et al.*, 1991). It was established that listeriosis was an important food-borne infection. *Listeria* microorganisms are well equipped to survive through food-processing technologies. For example, they tolerate high concentrations of salt and relatively low pH, and worst of all, they are able to multiply at refrigeration temperatures. This makes *Listeria* microorganisms a serious problem to food safety and the food industry should concern for it. The most frequently contaminated foods were soft cheeses, sausages, smoked fish, salads, and in general industrially produced, refrigerated ready-to-eat products that were eaten without cooking (McLauchlin *et al.*, 1990; Rocourt, 1996).

Researchers in immunology had been interested in *L. monocytogenes* since the pioneering work of Mackaness in the early 1960s, because cell mediated immunity to human listeriosis was easily reproducible in laboratory rodents and protection could be transferred in syngeneic mice through spleen cells. For decades, the experimental model of *Listeria* infection in mice has given a significant contribution to understand the cellular immune response. Intraperitoneal infection of *Listeria monocytogenes* to mice induced infection of the spleen. This bacterium has been used in immunological

research as a prototype intracellular pathogen (Shen *et al.*, 1998). Studies in murine listeriosis had revealed that control and elimination of *L. monocytogenes* in peripheral organs including spleen and liver as well as in the CNS were critically dependent on *L. monocytogenes*-specific CD4 and CD8 T cells (Kwok *et al.*, 2002). Activated CD8⁺ T cells produce perforin and TNF- α to inhibit *Listeria* growth. White *et al.* (2000) uncovered a pathway of CD8⁺ T cell-mediated antilisterial immunity in the liver that perforin and TNF- α worked independently. Hepatocytes might contribute to provide protection by becoming less permissive to intracellular proliferation of *Listeria* organisms upon exposure to IFN- γ co stimulated with other cytokines (Szalay *et al.*, 1995). Host's immunity determined the manifestation of clinical disease upon exposure to *L. monocytogenes*. Most listeriosis were found in humans with a physiological or pathological defect that affected cell-mediated immunity. Listeriosis was associated in most cases with at least one of the following conditions, such as malignancies, antineoplastic chemotherapy, immunosuppressant therapy (organ transplantation or

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corticosteroid use), chronic liver disease, and diabetes. The groups at risk for listeriosis were pregnant women, neonates, the elderly (55 to 60 years and older), and immunocompromised or debilitated adults with underlying diseases (Mc Lauchlin, 1990; Rocourt, 1996). The above reports supported that immunomodulators could help immunocompetent cells to kill the causative agent in listeriosis.

Several studies had shown that green tea catechin had immunomodulatory effects by regulating cytokines production. Epigallocatechin gallate (EGCG), a major form of tea catechin stimulated the production of interleukin-12 (IL-12), gamma interferon (IFN- γ), and tumor necrosis factor (TNF- α) in macrophages induced by *L. pneumophila* infection (Matsunaga *et al.*, 2001). EGCG induced production of IL-12, and TNF- α in macrophages that were suppressed by nicotine. EGCG diminished the immunosuppression of alveolar macrophages induced by cigarette smoke condensate (CSC). Treatment with EGCG also strengthened the resistance of macrophages to infection (Matsunaga *et al.*, 2002). Catechin extracted from another herb, *Spatholobus suberectus* Dunn could stimulate the proliferation of hematopoietic progenitor cells in bone marrow depressed mice by regulating IL-6 mRNA and GM-CSF mRNA expressions (Chen *et al.*, 2005). This recent study was proposed to investigate further immunomodulatory effect of GTE based on previous studies.

MATERIALS AND METHODS

Balb/c mice were obtained from "Pusat Veterinaria Surabaya" which fulfilled the following characteristics: male, six weeks old, 20 - 25 gram of weight, and without any abnormalities. The green tea polyphenon-60, EGCG, and ECG were purchased from Sigma Chemical Company.

The mice were acclimated for one week and given free access to water and a commercial stock diet (CP 511, Pokphand). They were randomly divided into 11 groups, each group consisted of five mice. Group I mice were not given green tea nor inoculated with bacteria. Group II mice were not given green tea but they were inoculated with *L. monocytogenes*. Group III to V mice were treated with green tea polyphenon-60 in different doses (1.5 mg, 3 mg, and 6 mg) every day; Group VI to VIII mice were treated with EGCG (0.5 mg, 1 mg and 2 mg) every day. Group IX to XI mice were treated with EGC (0.15 mg, 0.3 mg and 0.6 mg) every day. The mice in the group III to XI were treated with those compounds of green tea orally for 14 days. They

were inoculated with 10^4 *L. monocytogenes* intra peritoneally on the 10th day. All the mice were sacrificed on day 15th for counting the number of lymphoblasts in spleens.

RESULTS

The effects of green tea polyphenon-60 on lymphocytes proliferation

Green tea polyphenon-60 increased the number of lymphoblasts in mice inoculated with *L. monocytogenes* (Fig.1).

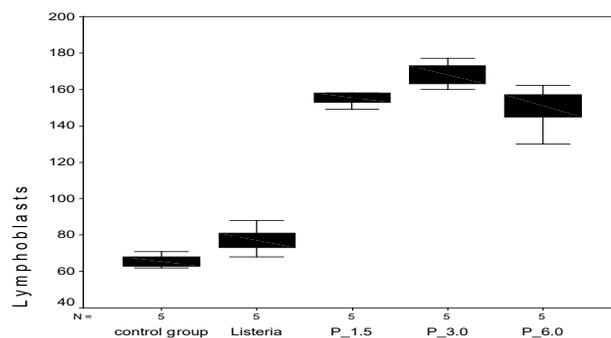


Fig.1 Box plot description of the amount of lymphoblasts from the control group and tea polyphenon-60 treated groups.

P_1.5 : mice were treated with 1.5 mg green tea polyphenon-60

P_3.0 : mice were treated with 3.0 mg green tea polyphenon-60

P_6.0 : mice were treated with 6.0 mg green tea polyphenon-60

Table 1. The p values between the control group and tea polyphenon-60 treated groups

Groups		p values
control	listeria	0.020
	P_1.5	0.000
	P_3.0	0.000
	P_6.0	0.000
listeria	P_1.5	0.000
	P_3.0	0.000
	P_6.0	0.000
P_1.5	P_3.0	0.008
	P_6.0	0.176
P_3.0	P_6.0	0.000

significant at $p < 0.05$

The effects of EGC on lymphocytes proliferation

EGC increased the number of lymphoblasts in mice inoculated with *L. monocytogenes* (Fig. 2).

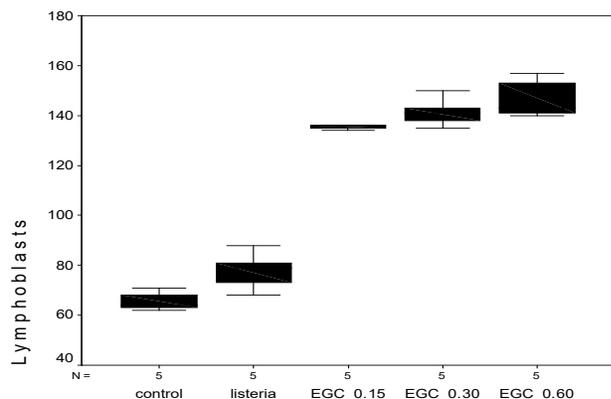


Fig. 2 Box plot description of the amount of lymphoblasts from the control group and EGC treated groups.

EGC_0.15 : mice were treated with 0.15 mg EGC

EGC_0.30 : mice were treated with 0.30 mg EGC

EGC_0.60 : mice were treated with 0.60 mg EGC

Table 2. The p values between the control group and EGC treated groups

Groups		p values
control	listeria	0.003
	EGC_0.15	0.000
	EGC_0.30	0.000
	EGC_0.60	0.000
listeria	EGC_0.15	0.000
	EGC_0.30	0.000
	EGC_0.60	0.000
EGC_0.15	EGC_0.30	0.115
	EGC_0.60	0.005
EGC_0.30	EGC_0.60	0.153

significant at $p < 0.05$

The effects of EGCG on lymphocytes proliferation

EGCG increased the number of lymphoblasts in mice inoculated with *L. monocytogenes* (Fig. 3).

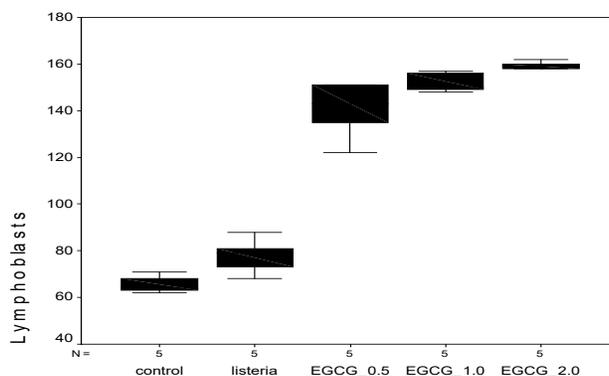


Fig.3 Box plot description of the amount of lymphoblasts from the control group and EGCG treated groups.

EGCG_0.5 : mice were treated with 0.5 mg EGCG

EGCG_1.0 : mice were treated with 1.0 mg EGCG

EGCG_2.0 : mice were treated with 2.0 mg EGCG

Table 3. The p values between the control group and EGCG treated groups

Groups		p values
control	listeria	0.012
	EGCG_0.5	0.000
	EGCG_1.0	0.000
	EGCG_2.0	0.000
listeria	EGCG_0.5	0.000
	EGCG_1.0	0.000
	EGCG_2.0	0.000
EGCG_0.5	EGCG_1.0	0.016
	EGCG_2.0	0.000
EGCG_1.0	EGCG_2.0	0.109

significant at $p < 0.05$

The effects of green tea polyphenon-60, EGC, and EGCG on lymphocytes proliferation.

Green tea polyphenon-60 induced the highest effect to increase the number of lymphoblasts in mice inoculated with *L. monocytogenes* (Fig. 4).

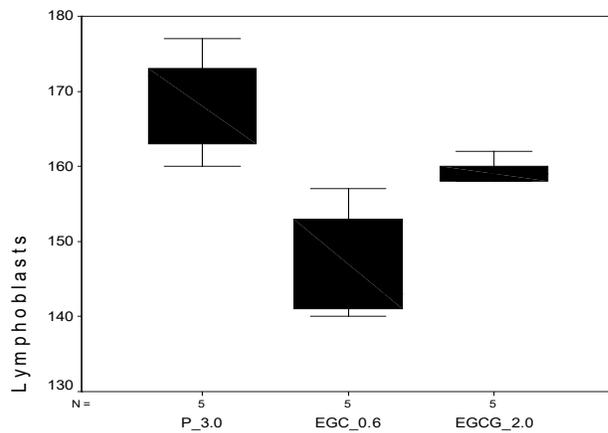


Fig. 4 Box plot description of the amount of lymphoblasts from the tea polyphenon-60, EGC, and EGCG treated groups.

Table 4. The p values between the tea polyphenon-60, EGC, and EGCG treated groups

Groups		p values
tea polyphenon-60	EGC	0.000
	EGCG	0.030
EGC	EGCG	0.07

significant at $p < 0.05$

DISCUSSION

Previous studies in murine listeriosis had revealed that control and elimination of *L. monocytogenes* in peripheral organs including spleen and liver as well as in the CNS were critically dependent on *L. monocytogenes*-specific CD4 and CD8 T lymphocytes (Kwok *et al.*, 2002; White *et al.*, 2000). In mice, CD4 and CD8 T cells are mainly directed against listeriolysin (LLO) and the p60 protein of *L. monocytogenes*. Immunodominant CD4 (LLO₁₉₀₋₂₀₁) and CD8 (LLO₉₁₋₉₉) as well as subdominant CD4 (p60₃₆₇₋₃₇₈) and CD8 (p60₂₁₇₋₂₂₅) epitopes against these proteins have been identified (Szalay *et al.*, 1995). Kwok *et al.* (2002) showed that specific T cells could exist in the brain and were able to slow down bacterial replication in cerebral listeriosis. The infection of peripheral lymphatic organs by *L. monocytogenes* and the presence of *Listeria*-specific CD4 and CD8 T cells in the spleen suggested that intracerebral *Listeria*-specific T cells were primed and activated in peripheral lymphatic organs and were subsequently recruited to the brain.

Previous study had reported that catechin extracted from another herb, *Spatholobus suberectus* Dunn could stimulate the proliferation of hematopoietic progenitor cells in bone marrow depressed mice (Chen *et al.*, 2005). In this study, it was proven that increase number of lymphoblasts was observed in all groups of mice treated with green tea polyphenon-60, EGCG, and EGC. Green tea polyphenon-60 had the highest effect to increase the number of lymphoblasts in mice inoculated with *L. monocytogenes*.

CONCLUSION

It was concluded that green tea extracts could induce lymphocyte proliferation. The green tea polyphenon-60 (the mixture of tea polyphenols) was more effective than that of pure EGCG or EGC alone when it was used to increase the number of lymphoblasts. It is recommended to explore further the effect of green tea extract in humans treated with antineoplastic chemotherapy that raises immunosuppressant effects.

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