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# Effects of Green Tea Catechin on Serum Lipid Composition and Angiotensin-Converting Enzyme Activity in Rats Exposed to Microwave

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## Abstract

**This study aims to investigate the changes in the lipid composition and angiotensin-converting enzyme activity in serum of rats exposed to microwave radiation, and to examine improving effects of green tea catechin to the lipid composition. The microwave-exposed rats received the normal and one of 3 diets: catechin free (MW-0C), 0.25% catechin (MW-0.25C) or 0.5% catechin (MW-0.5C). Rats were sacrificed 6th day after microwave radiation (2.45 GHz, 15 min). The concentration of serum triglyceridein MW-0C group was increased by 85%, compared with the normal group, but that of MW-0.25C group with 0.25% catechin supplementation was to 17% lower, compared with the MW-0C group. There was no significant difference between normal group and MW-0.5C group. Total-cholesterol and LDL-cholesterol concentrations were increased by 21.4% and 38.6%, respectively, by microwave irradiation. The concentration of HDL-cholesterol in MW-0C group was lower to 29.3%, but it was maintained at the normal level by catechin supplementation. There was no significant difference among four groups in HLD-cholesterol/LDL-cholesterol. Atherogenic index in MW-0C group was increased by 56%, compared with the normal group. Angiotensin-converting enzyme (ACE) activity in lung tissue of MW-0C group was increased by 59%, while that of MW-0.25C and MW-0.5C group were maintained at the normal level. ACE activity of MW-0C group in serum was increased by 122.8%, compared with the normal group. Catechin supplementation group was significantly reduced, compared with the MW-0C group ACE activity. In conclusion, microwave irradiation increased the serum triglyceride and cholesterol concentrations and ACE activity which are considered as the blood pressure increasing agents. However, catechin supplementation decreased the level of triglyceride, total cholesterol, LDL cholesterol, and ACE activity, which maybe consider catechin as being agent of lowering effect for blood lipid profile for atherogenesis.**

**Key words:** microwave, green tea catechin, serum lipid composition, angiotensin converting enzyme

## INTRODUCTION

The microwaves which contain high frequencies involve a substantial amount of energy, and exposure to microwaves has been found to have a harmful effects on actively dividing cells, such as reproductive organs, cardiac and pulmonary tissues, and white blood corpuscles. Thus, an exposure to microwaves is associated with various morbidities (1,2). Based on the analyses of preceding basic researches (3-6), it is likely that when living body was exposed to microwave, antioxidative defense system was weakened and the lipid peroxide increased, which was caused by oxidative toxicity generated from reactive oxygen species.

Microwave toxicity is caused by reactive oxygen species (ROS) which was generated during oxidation (3-6). Thus, microwave toxicity induced abnormal lipid composition and accelerated the several chronic disease, such as hypertension, artherosclerosis and hyperlipidemia. Especially, hypertention was prevalent in human and animal to whom microwave was exposed and it was influenced from angiotensin-converting enzyme (ACE) activity, generated angiotensin II, act as increasing blood pressure. Jauchen and Frei (7) and Ryan (8) observed changes in the respiratory rate, heart rate and an increase in arterial blood pressure in microwave-irradiated rats.

On the other hand, catechin, one of the polyphenol compound which is found in green tea, previously has been reported to have pharmalogical effects (9-11), including antioxidation. Especially, it is well known that catechins have preventing effect of adult disease, such as artherosclerosis and hyperlipidemia, since catechin suppressed the formation of lipid peroxide and cholesterol level in the living body (12,13). Yang et al. (14) reported that ingredients prepared from green tea reduced the serum cholesterol level in rats. Also, it was reported (15,

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16) that (-)-epigallocatechin gallate and (-)-epicatechin gallate prepared from green tea reduced the serum cholesterol level in rats with high-cholesterol diet, and increased the HDL-cholesterol by epicatechin tube feeding. Also Choi et al. (17) reported that an increased ACE activity in chronic cadmium-exposed rats and confirmed the suppressing effect of blood pressure increasing and indicated that the cause was the suppressed the ACE activity by dietary catechin supplementation. Cho et al. (18) also reported that flavonoid compound which was prepared from green tea acted as a strong inhibiting effect to ACE activity.

Accordingly, this study aims to elucidate the changes in the lipid composition and ACE activity and to examine the lipid composition-improving effects of green tea catechin in serum of the microwave-irradiated rats.

## MATERIALS AND METHODS

### Experimental animals and diets

Male Sprague-Dawley rats weighing about 100 g were purchased from KRITC (Korea Research Institute of Chemical Technology) (Daejon, Korea). The animals were individually housed in stainless steel cage in a room with controlled temperature (20~23°C). The rats were adjusted for one week and then allotted to one normal group and three level of microwave expose groups: microwave exposed groups were divided into three groups [catechin free diet (MW-0C) group, 0.25% catechin (MW-0.25C group) and 0.5% catechin (MW-0.5C) group] according to the level of dietary catechin supplementation. The four animal groups were fed experimental diet for 4 wks. The experimental animal number was 10 per groups (Table 1). Crude catechin powder from green tea was prepared by the method of Matsuzaki and Hata (19). Crude catechin purity was 87.5% (Table 2). And the experimental design was approved by the committee of Catholic University of Daegu for care and use of laboratory animals.

**Table 1.** Classification of experimental groups

| Groups[1)] | Catechin (% of diet) | Microwave |
|---|---|---|
| Normal | 0 | - |
| MW-0C | 0 | + |
| MW-0.25C | 0.25 | + |
| MW-0.5C | 0.5 | + |

Irradiated 2.45 GHz microwave for 15 min.
[1)]Normal: No microwave irradiation.
MW-0C: Microwave irradiation, catechin free diet.
MW-0.25C: Microwave irradiation, catechin supplementation (0.25% catechin diet).
MW-0.5C: Microwave irradiation, catechin supplementation (0.5% catechin diet).

### Instrument and methods for microwave irradiation of experimental animals

The experimental instrument used for a high frequency generator was designed to be available for a remote control to optionally adjust both output and exposure time by remodeling a household electronic oven with 2.45 GHz continuous wave (CW) microwave. The incident power density was measured using an America Hewlett Packard's EMC analyzer (8594EM). The power density was measured using the 35cm distance of the Horn Antenna (EMCO 3115) as an exposure point, after adjusting the distance several times for maximum dose-rate to be about 15 minutes. The average whole-body, specific absorption rate (SAR) was measured using a calorimetric technique. The SAR was determined to be 9.2 W/kg for an incident power density of 40 $mW/cm^2$.

Experimental animals were sacrificed at 6th day after microwave irradiation, since the previous research (3) has shown that oxidative damage was the severest at 6th day after microwave irradiation.

### Preparation of serum

After blood was drawn from the artery of the abdominal region, the serum was obtained by centrifuging at 3000×g for 10 minutes. The serum were stored at 70 °C after being fast frozen (20). Concentrations of triglyceride and cholesterol in serum were measured. For measurement of triglyceride in serum, standard enzyme colorimetry method kit (Asan Co., Korea) was used for measuring the absorbance at 550 nm and then calculated the concentration of triglyceride in serum. For measurement of serum total cholesterol, standard enzyme colorimetry was used for measuring the absorbance at 500 nm and total concentration of cholesterol was calculated. For measuring HDL-cholesterol, 2% detron sulfate and 1 M $MgCl_2$ precipitation solution (1:1) were added and standard enzyme at 500 nm. LDL-cholesterol was calculated by Friedwald formula (21) [Total cholesterol - (HDL cholesterol + TG/5)] and atherogenic index (22) was calculated with [Total cholesterol - HDL cholesterol] / [HDL cholesterol] formula.

### Angiotensin-converting enzyme

The activity of angiotensin-converting enzyme in serum

**Table 2.** Composition of crude catechin powder from green tea

| Catechin (μg)/100 μg powder | EGC[1)] | EC[2)] | EGCG[3)] | ECG[4)] | Total |
|---|---|---|---|---|---|
| | 24.2±0.024 | 7.0±0.013 | 45.38±0.06 | 10.92±0.06 | 87.50 |

[1)](-)EGC: Epigallo catechin. [2)](-)EC: Epicatechin. [3)](-)EGCG: Epigallo catechin gallate. [4)](-)ECG: Epicatechin gallate.

and lung, was measured by the method of Hugo et al. (23).

### Statistical analysis

Result were analyzed by ANOVA and Tukey's Honestly Significant Difference test if the statistical significance was found from ANOVA. Difference were considered significant at $p<0.05$.

## RESULTS

### Serum triglyceride concentrations

The concentration of serum triglyceride in MW-0C group was increased by 85%, compared with the normal group, but that of MW-0.25C group with 0.25% catechin supplementation was to 17% lower, compared with the MW-0C group. There was no significant difference between MW-0.25C group and MW-0.5C group (Fig. 1).

### The concentration of total cholesterol, HDL cholesterol, LDL cholesterol and atherogenic index

Total-cholesterol and LDL-cholesterol concentrations were increased by 21.4% and 38.6% in MW-0C group, respectively, by microwave irradiation. The concentration of HDL-cholesterol in MW-0C group was lower to 29.3% compared with normal group, but it was maintained at the normal level by catechin supplementation, thus atherogenic index was improved (Fig. 2).

There was no significant difference among four dietary groups in HDL-cholesterol / LDL-cholesterol. Atherogenic index in MW-0C group was increased by 56%, compared with the normal group. MW-0.25C and MW-0.5C group were lower to 25% and 19%, respectively, compared with the MW-0C group (Fig. 3).

### Activity of angiotensin-converting enzyme (ACE) in serum and lung tissue

The results of angiotensin-converting enzyme activity

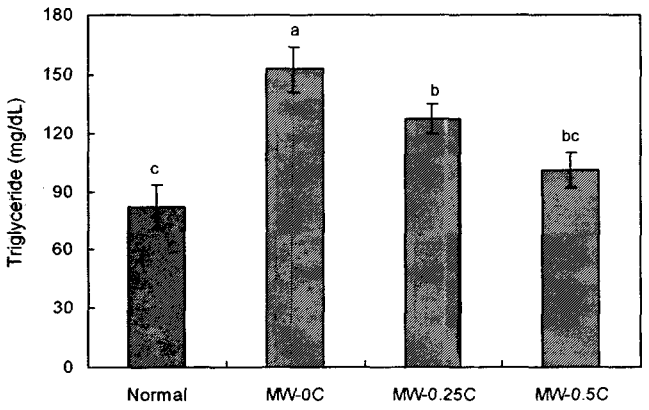


**Fig. 1.** Effect of green tea catechin on serum triglyceride concentration in rats exposed to microwave. All values are the mean ± SE (n=10). Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test. The experimental conditions are the same as Table 1 & 2.

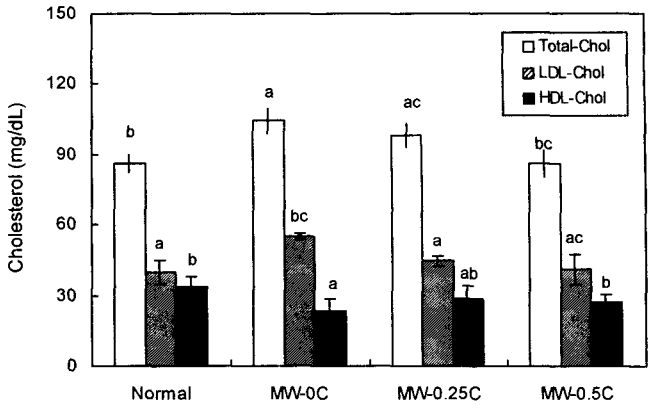


**Fig. 2.** Effect of green tea catechin on serum total-cholesterol, HDL-cholesterol and LDL-cholesterol concentration in rats exposed to microwave. All values are the mean ± SE (n=10). Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test. The experimental conditions are the same as Table 1 & 2.

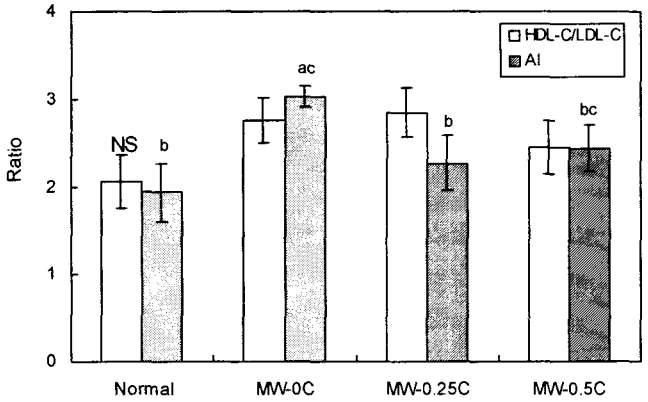


**Fig. 3.** Effect of green tea catechin on serum HDL-chol / total-chol, atherogenic index in rats exposed to microwave. All values are the mean ± SE (n=10). Values with different superscript letters are significantly different at $p<0.05$ by Tukey's test. The experimental conditions are the same as Table 1 & 2.

are shown on Fig. 4. In lung tissue, ACE activity in MW-0C was increased by 59%, while that of MW-0.25C and MW-0.5C group was maintained the normal level. Thus, it seemed that the catechin has the suppressing effect of the ACE activity. In serum, that of MW-0C group was increased by 122.8%, compared to the normal group. Catechin supplementation group was significantly reduced, compared with the MW-0C group (Fig. 4).

## DISCUSSION

The current study was conducted to observe the improving effect of green tea catechin to lipid composition in microwave-exposed rats. Microwave-exposed rats with or without supplemental green tea catechin were observed on lipid composition in serum and ACE activity in serum and lung tissue.

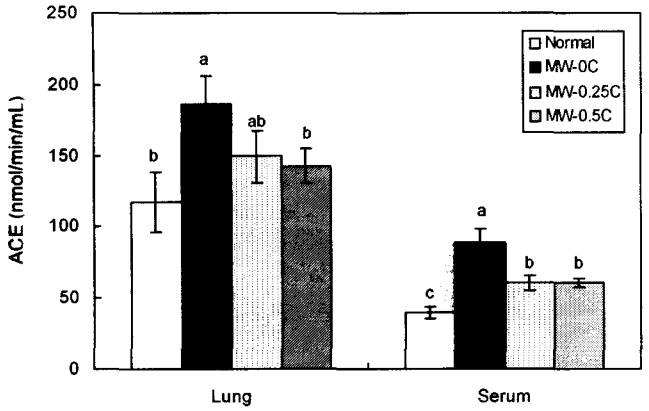


**Fig. 4.** Effect of green tea catechin on angiotensin-converting enzyme (ACE) activity in lung and serum in rats exposed to microwave. All values are the mean ± SE (n=10). Values with different superscript letters are significantly different at $p < 0.05$ by Tukey's test. The experimental conditions are the same as Table 1 & 2.

In this study, The concentration of serum triglyceride in MW-0C group was increased by 85%, compared with the normal group, but that of MW-0.25C group with 0.25% catechin supplementation was to 17% lower, compared with the MW-0C group. There was no significant difference between normal group and MW-0.5C group. However catechin-supplemented group maintained the normal level in serum triglyceride. The results of this study agree with those of Choi et al. (24) who reported that catechin reduced the triglyceride in rats with hyperlipidemia. Thus, it seemed that microwave irradiation increased oxidative stress and influenced serum lipid composition and antioxidative capacity of catechin improved this changes. In this study, total-cholesterol and LDL-cholesterol concentrations were increased by 21.4% and 38.6%, respectively, by microwave irradiation. The concentration of HDL-cholesterol in MW-0C group was lower to 29.3%, but it was maintained at the normal level by catechin supplementation, thus atherogenic index was improved. This result agrees with those of Kwan et al. (15) who reported that (-)-epigallocatechin gallate and (-)-epicatechin gallate supplementation reduced the serum cholesterol level in high cholesterol diet. Thus, it seemed that catechin improved the serum cholesterol composition by accelerating the cholesterol metabolism caused by microwave irradiation.

In this study, There was no significant difference among four groups in HDL-cholesterol / total-cholesterol. Atherogenic index in MW-0C group was increased by 56%, compared with the normal group. Atherogenic index in MW-0.25C and MW-0.5C group were lower to 25% and 19%, respectively, compared with the MW-0C group. The possible reason for atherogenic index lowered by catechin supplementation is likely that HDL-cholesterol were increased and total-cholesterol and LDL-cholesterol were reduced, thus, the utilization of LDL-cholesterol was accelerated. In this study and other studies, it is likely that abnormal lipid metabolism by microwave irradiation was caused by reactive oxygen species (ROS) and oxidative toxicity which were generated during oxidation. And catechin supplementation improved the changes of lipid composition. In this study on ACE activity in lung tissue, that of MW-0C was increased by 59%, and those of MW-0.25C and MW-0.5C group were maintained at the normal level. Thus, it seemed that the catechin has the suppressing effect of the ACE activity. In serum, ACE activity of MW-0C group was increased by 122.8%, compared to the normal group. Catechin supplementation group was significantly reduced, compared with the MW-0C group.

Kameda et al. (25) and Fukuyama and Hikino (26) reported that flavonoid ingredients prepared persimmon leaf has suppressing effect of ACE. Cho et al. (18) reported that flavon-3 compound prepared from green tea has suppressing effect of ACE. The results of this study were similar to Choi's study (17) which reported that ACE activity was increased due to increasing the free radical generation and oxidative stress in chronic cadmium poisoned rats, but catechin supplementation normalized the lipid composition.

In conclusion, microwave irradiation increased the serum triglyceride and cholesterol concentrations and ACE activity which are considered as the blood pressure increasing agents. However, catechin supplementation decreased the level of triglyceride, total cholesterol, LDL cholesterol, and ACE activity, which may be considered catechin as being agent of lowering effect for blood lipid profile for atherogenesis.

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