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Inhibitory Effect of Jeju Tea Extracts and Vanadate on Postprandial Hyperglycemia and Hypertension, and *In Vitro* Study

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제주산 녹차, 발효차 추출물과 바나듐의 식후 혈당강하 및 항고혈압 기능에 미치는 *In Vitro* 효과

박신영

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ARTICLE INFO	ABSTRACT		
Received November 6, 2020 Revised November 25, 2020 Accepted December 4, 2020	The inhibitory effect on α -glucosidase, a marker of postprandial hyperglycemia, and angiotensin- converting enzyme (ACE), a marker of hypertension, was analyzed using non-fermented green tea and three different types of fermented tea, which are popular beverages in modern life. Green tea was mixed with trace amounts of vanadate (50 µg/mL), which has insulin-mimetic effects, to investigate the synergistic effect of vanadate on the inhibition of α -glucosidase. The concentration of epigallocatechin gallate (EGCG) and caffeine was also checked. The extracts of green tea and fermented teas showed clear inhibition on α -glucosidase, which caused a decrease in the		
Key words Angiotensin converting enzyme Fermented tea Green tea Postprandial glucose Vanadate	postprandial glucose levels. The inhibitory effect was most prominent in the 20% fermented tea. Trace amounts of vanadate (50 μg/mL)-mixed green tea extract had twice the inhibitory effect on α -glucosidase than the pure tea extract. All teas showed inhibitory effects on ACE. Among those, the effect was most prominent in green tea, which had higher concentrations of EGCG. In contrast, the postprandial glucose-lowering effect and ACE inhibition of the fermented teas, which have a lower level of EGCG, was attributed to some other different functional substances.		

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INTRODUCTION

Hypertension, hyperlipidemia, diabetes are the most representative chronic disorder, with a prevalence rate of 89.2% in the elderly people over 70 years old [1]. According to the National Health Interview Survey (NHIS) data, 2.6 million Koreans have both hypertension and dyslipidemia, 0.72 million have both diabetes and dyslipidemia, 0.64 million have both hypertension and diabetes, and 1.4 million have all three diseases. Simultaneously having multiple of these risk factors exponentially increases cardiovascular risk compared to having only a single risk factor [2, 3]. The prevalence of diabetes among adults 30 years or older was 14.4% (15.8% in men and 13.0% in women) when the HbA1c criterion was included in the definition of diabetes. The prevalence of diabetes was even higher (29.8%) when the analysis was limited to adults older

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than 65 years, meaning that one in three Koreans 65 years or older had diabetes [4]. The development rate of diabetes is higher by 2.5 times in hypertensive patients, with 70% of diabetic patients concomitantly suffering from hypertension. Since suffering from both disorders may affect an individual's health more severely than compared to having each disorder separately, the importance of hypertension management in prevention against diabetes must be acknowledged. As there are reports on the development of myocardial infarction with the increase of plasma angiotensin converting enzyme (ACE) in hypertensive patients [5]. A recent dose-response meta-analysis of prospective studies reported that fruit and/or vegetable consumption had a beneficial effect like those significant decreases in the risk of coronary heart disease, stroke, and cardiovascular disease (with risk reductions ranging from 8% to 21%) [6, 7]. ACE inhibitors are used for the management of essential hypertension. Recently, interest in functional food products without many side effects with the prolonged period of usage has grown between people with mild hypertension who do not necessarily require anti-hypertensive prescription and those who are near the cut-line of hypertension diagnosis, for prevention of hypertension-related disorders. Along with the studies that have identified the inhibition of ACE and renin activation in polyphenol rich food, there have been recent anti-hypertensive activation report of tomato extracts and Guava leaves through ACE inhibition [8, 9].

Interest in future medicine has changed from a simple nutritional aspect of the food and water we take, to a higher level for functional health such as biophylaxis, prevention of disorders, recovery, and anti-aging. Thus, it is important that we choose our favored food and daily drinking water considering its preventative function. Habitual and reasonable amount of drinking on a daily basis is considered the most effective way for prevention against disorder. Dried leaves of *Camellia sinensis* v. sinensis, especially known for its refreshing scent at its natural state, is not only

often widely used for its specific scent developed during its manufacturing process, but is also known to be effective in prevention of acute myocardial infarction with its high flavonoid concentration through inhibition against cardiovascular related-lipid peroxidation and arteriosclerosis. Furthermore, with the known physiological function of lipid peroxidation inhibition and high antioxidation of EGCG; highest content of catechin in green tea, EGCG is the most optimized favored food for prevention against chronic disorders [10–12].

Heyliger et al [13] report on the hypoglycemic function of sodium metavanadate in streptozotocin (STZ) induced diabetic mouse, in a study using vanadium containing underground water of Mount Fuji in 1985, various studies had been further conducted. Content of vanadium in natural underground water varies for each region. Underground water around Mount Fuji contains a relatively high content of vanadium at 100 µg/L, while Jeju Island has also been reported to contain around 25~30 mg/L of vanadium in its underground [14, 15]. Such hypoglycemic function of vanadium was induced from activation of the insulin receptor and sugar delivery system. When vanadium containing natural underground water of Mount Fuji $(100 \,\mu\text{g/L})$ was administered for 4 months continuously in human, it inhibited the rise of post-meal blood sugar, proving its effect as a supplemental measure rather than a treatment. In a research on in vivo accumulation of vanadium in animals during the same 4 month period, not much of a difference was observed within the major organs such as the liver and the spleen compared to the administered concentration, proving its low in vivo accumulation [16, 17].

To evaluate the anti-hypertensive and anti-diabetic effect of Jeju green tea and fermented tea extract, ACE inhibition for blood pressure control and α -glucosidase inhibition for post-meal blood sugar rise were measured. Moreover, with the known vanadium content in natural underground Jeju water (10~30 mg/L) as the baseline [15] sufficient concentration (50 µg/mL) for result

deduction was treated together with green tea and fermented tea extract. This process was conducted to study the synergistic effect of α -glucosidase inhibition when treated together with vanadium compared to individual effect of green/fermented tea, as vanadium shows similar activity to insulin. Thus, this study also aimed on the possibility of synergistic effect by daily intake of green/fermented tea with natural vanadium containing underground Jeju water to control postprandial hyperglycemia.

MATERIALS AND METHODS

1. Extraction method of green tea and fermented tea

Four types of teas were provided by regional tea plantation for extraction; not fermented green tea (G) and fermented tea at its different fermentation stages 20% fermented tea (F20), 50% fermented tea (F50), and 80% fermented tea (F80). To manufacture tea extracts, 500 g of the dried tea leaf were soaked in 90°C water for 1 hour. Using the one time extracted tea leaf, extraction was performed for 4 times with the same method. The collected extraction were filtered and compressed for concentration under vacuum decompressor, and lyophilized for be used in this study [12]. All analytical reagents used in the study were Sigma Chemical Co., (St Louis, MO, USA), Aldrich Chemical Co., (Steineheim, Germany) and Merck Ltd., Darmstadt (Germany).

2. Postprandial hypoglycemic effect

To evaluate postprandial hypoglycemic effect, α glucosidase inhibitory activity was measured under two different conditions [18]. First of all, individual α glucosidase inhibitory activity of the 4 types of tea extracts were measured. Afterwards, green tea extract concentrations were mixed with 50 µg/mL vanadium to measure the synergic effect of the tea extract with vanadium. α -glucosidase enzyme was mixed with 200 mM potassium phosphate buffer (pH 6.8) to be added to the green tea ferment extract. It was reacted at 37°C for 10 minutes, prior to the addition of 3 mM ρ -nitrophenyl α -glucopyronoside (ρ -NPG) dissolved in 0.1 M phosphate buffer (pH 7.0) for additional 10 minute reaction. 0.1M Na₂CO₃ was added to stop the reaction. Absorbance was measured at 405 nm to calculate the % inhibitory activity (1). dH₂O was used as a negative control absorbance to calculate the inhibitory activity of the enzyme. Acarbose, currently marketed post-prandial hypoglycemic agent, was dispensed to 10 mg/mL as a positive control of the inhibitory activity evaluation.

Inhibition activity (%) =
$$\left(1 - \frac{\text{ABS}_{\text{control}} - \text{ABS}_{\text{sample}}}{\text{ABS}_{\text{control}}}\right) \times 100$$
(1)

 $ABS_{control}$: absorbance at 405 nm determined with dH_2O instead of tea extracts or acarbose,

 ABS_{sample} : absorbance at 405 nm determined with tea extracts or acarbose.

3. Anti-hypertensive activity

ACE, often used as an anti-hypertensive activity index, is an enzyme that converts inactive angiotensin I to an active angiotensin II to elevate the blood pressure. Applying the method by Cushman and Cheung [19], inhibitory effect of ACE was tested using Hippuryl-L-histidyl-Leucine (HLL) as the substrate of the enzyme. Tea extracts of each concentration were added along with sodium phosphate buffer 100 µL and HHL 50 µL. Each was reacted at 37°C for 10 minutes. Rabbit lung acetone powder 100 µL was added and left to react for 30 minutes for second reaction. To stop further enzyme reaction, 1N HCl 350 µL was added for hippuric acid extraction, and absorbance was measured at 228 nm. Distilled water was added to the same volume in place of tea extract for the control, and no enzyme was added for the blank. Activity of the control was set as 100% according to the equation below (2), to calculate the inhibitory activity %.

Inhibition activity (%) =
$$\left(1 - \frac{ABS_{sample} - ABS_{blank}}{ABS_{control}}\right) \times 100$$
(2)

ABS_{sample}: absorbance at 228 nm determined with tea extracts,

 ABS_{blank} : absorbance at 228 nm determined with dH_2O instead of enzyme,

 $ABS_{control}$: absorbance at 228 nm determined with dH_2O instead of tea extracts.

4. Caffeine and EGCG concentration measurement with HPLC

Caffeine and EGCG concentration of the 4 types of tea extracts were measured using HPLC (Shimadzu model LC-2010, Japan). SHIM-PACK (VP-005, 150×4.6) of Shimadzu was used for HPLC column. UV270 nm, at a flow rate of 1 mL/min was used for the detection. Standard caffeine and EGCG concentrations were prepared to 200 μ g/mL, and were serial diluted to 5, 10, 50, 100, 200 μ g/mL each for evaluation. Detection was conducted for 3 times, and all measurement confirmed to be acceptable with R2 value of all above 0.999.

5. Statistical analysis

standard deviation of mean (N=3).

All test results were conducted more than 3 times.

Average and standard deviation for all 3 test results were calculated as a mean \pm SD (N=3). Graphad Prism (GraphPad Software Inc., La Jolla, CA, USA) was used for statistical calculations. One-way ANOVA for repeated measures was performed followed by Dunnett's post hoc test or Bonferroni's post hoc test. Statistical significance was taken at *P*<0.05.

RESULTS

1. Postprandial hypoglycemic effect evaluation of tea extract

The inhibitory activity of α -glucosidase obtained in this study by the extracts of the 3 types of fermented tea and green tea was mostly dose-dependent. Figure 1 shows the measurement results of α -glucosidase inhibition to prevent sugar decomposition. The inhibitory activity was observed with the increase of extract concentration, 10, 20, 40, 60, 80 and 100 µg/mL.

F20 showed the most superior inhibitory effect of over 90% at 100 μ g/mL concentration, however the inhibitory activity of G was lower than fermented teas at

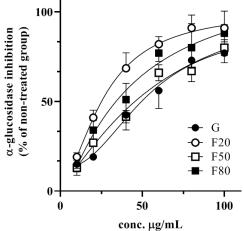


 Figure 1. Dose-dependent effect of four types tea extract on α

 glucosidase inhibition activity. The data were presented as mean ±

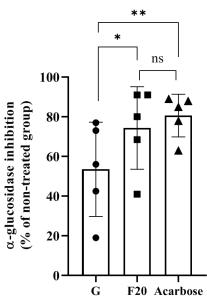


Figure 2. Inhibitory effects of acarbose and two types of tea extracts on α -glucosidase activity. Each dot represents the result of treated concentration: 20, 40, 60, 80 and 100 µg/mL. The data were presented as mean±standard deviation of mean (N=3). Significant differences determined by one-way ANOVA (*P<0.05, **P<0.01).

most concentrations. In order to identify the fermented tea effect for α -glucosidase inhibitory activity, the inhibitory activity of F20 and non-fermented green tea were compared with acarbose, which was used as a positive control (Figure 2).

Evaluation of α-glucosidase inhibitory activity of tea extract and vanadium mixture

The interesting finding was observed when the lower concentration of 50 µg/mL green tea extract was combined with vanadium. It showed significant increase of up to 90% inhibition compared to the inhibitory effect of singly treated green tea of 50%. F20, which showed the highest inhibitory rate when at single use, also showed significant increase in combination with vanadium, but the most notable α -glucosidase inhibition was observed in green tea and vanadium combination (Figure 3A). When the concentration of the tea extract was increased to double the initial concentration of 50, to 100 µg/mL, increase of the inhibitory rate was observed with the concentration increase, but did not show significance of synergistic effect with vanadium as it did in the lower concentration. F20 showed high enzyme inhibitory effect, but showed not much difference in combination with vanadium (Figure 3B). No enzyme inhibitory effect was observed in both F50 and F80 at both its single use or in vanadium combination.

3. Anti-hypertensive activity of the tea extract

Inhibitory activity of ACE for the non-fermented type green tea (G) and 3 different types of fermented tea (F20, F50, F80) were studied using different extraction concentrations, 0.08, 0.16, 0.31, 0.62, 1.25 and 2.50 mg/mL (Figure 4). ACE inhibitory activity increased rapidly with the increased concentration in all types of extract concentrations including green tea, confirming its superior anti-hypertensive biological activity even at its low concentration. α -glucosidase inhibitory activity of green tea was observed to be lower than F20,

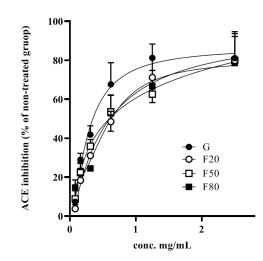


Figure 4. Inhibition activity against angiotensin converting enzyme (ACE) of four types of tea extract. Each dot represents the result of treated concentration: 0.08, 0.16, 0.31, 0.62, 1.25 and 2.5 mg/mL. The data were presented as mean±standard deviation of mean (N=3).

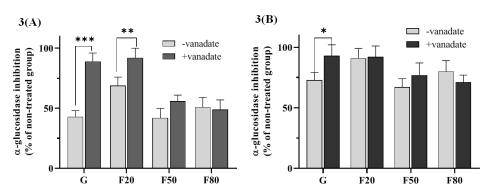


Figure 3. (A) α -glucosidase inhibitory rate (%) of single 50 μ g/mL concentration green tea extract (-vanadate) and combination with 50 μ g/mL vanadium (+vanadate). *P<0.05, **P<0.01, ***P<0.001. The data were presented as mean±standard deviation of mean (N=3). (B) α -glucosidase inhibitory rate (%) of single 100 μ g/mL concentration green tea extract (-vanadate) and combination with 50 μ g/mL vanadium (+vanadate). *P<0.01, ***P<0.01, **P<0.01, **P

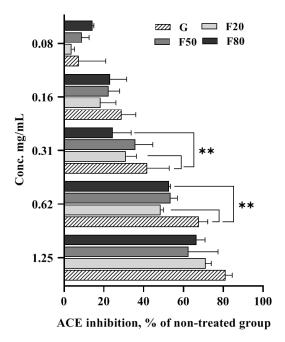


Figure 5. Inhibition activity against angiotensin converting enzyme (ACE) of four types of tea extracts. The data were presented as mean \pm standard deviation of mean (N=3). Significant differences determined by one way ANOVA (**P<0.01).

but was observed to be the most superior in ACE inhibition, showing 60% inhibition at 0.64 mg/mL concentration and above 80% inhibition at 1.25 mg/mL concentration. The 3 fermented tea extracts showed above 60% inhibition at an approximate 1.25 mg/mL concentration, confirming green tea to show the highest inhibitory effect. There was significant difference between non fermented tea (G) and fermented tea (F20, F50, F80) with respect to the ACE inhibitory activity at the different concentrations, especially at 0.31 and 0.62 mg/mL (Figure 5).

4. Caffein and EGCG content in green tea and fermented tea

Most polyphenol contents in green tea are flavonoids known as catechin. EGCG is known to take up to 30% of dried green tea form all catechin. The total content of EGCG and caffeine in green tea of Jeju Island and the 3 types of fermented tea were researched (Table 1). The EGCG content showed great difference between green tea and the 3 types of fermented tea. EGCG concen-

Table 1. Caffeine and EGCG content in green tea and 3 types of fermented tea (mg/L)

mg/L –	Tea types				
	G	F20	F50	F80	
Caffeine EGCG	$228\pm0.3^{1)}$ $225\pm2.2^{2)_{a}}$	203±0.6 49±0.1°	182±0.7 60±1.2 ^b	259±0.3 2.8±0.0 ^d	

 $^{1)}Values$ are presented as mean $\pm \,SD.$

²Values with different small letters in superscripts within a row are significantly different at ****P*<0.001 by Dunnett's multiple comparisons test in one-way ANOVA.

tration was observed to be 4 times higher in green tea compared to F20 and F50. Moreover, as hardly any EGCG was detected in F80, the biological activity of fermented tea was from a different material from that of catechin in green tea.

DISCUSSION

As an enzyme in the small intestine, α -glucosidase hydrolyzes carbohydrates such as disaccharides or polysaccharides into final digestive products such as monosaccharide like glucose for better absorption. Hydrolyzed monosaccharides by a-glucosidase in the small intestines are mostly rapidly absorbed in the upper small intestine to induce rapid elevation of postprandial blood sugar. α-glucosidases such as maltase or sucrase within the small intestines in normal people, are known to be appropriately inhibited to prevent rapid elevation of blood sugar [20]. Therefore, in order to reduce serum glucose levels and manage related diseases, a-glucosidase inhibition has been proposed as a suitable approach. This research investigated the enzyme inhibition ability of tea extracts to determine their potential utility as agent against α -glucosidase inhibitor for useful in the development of blood sugar related disorders such as type 2 diabetes. Acarbose showed 83% inhibition at 100 µg/mL concentration, showing a slightly lower inhibitory effect of F20 at the same concentration. G showed the lowest activity with a significant difference between the acarbose and F20. Similar results have also been reported in a study using Northern bamboo leaf extract [21]. Alpha-glucosidase inhibitory activity was observed to be the highest by 48.46% at 0.5 mg/mL ethyl acetate solvent layer in Northern bamboo leaf extract, showing a slightly higher inhibition acarbose of 40%. Despite such results, the reason for the main use of acarbose in Korea as postprandial hypoglycemic agent, is suspected to be because of its other relevant functions along with the simple α -glucosidase inhibitory effect [22]. However, the currently marketed postprandial hypoglycemic agents acarbose and voglibose have been reported to show an approximate 30% of gastrointestinal related side effects, such as abdominal distention and diarrhea. Thus, there are needs to find substitutional materials [20]. As a result of this study, we consider that natural extracts of green tea as well as the 3 types of fermented tea, may be used as functional materials in the future as anti-diabetic natural products.

As all 4 types of tea extracts were proved to have α glucosidase inhibitory effect as shown in Figure 1, vanadium (50 µg/mL); which is known to have an anti-diabetic effect, was mixed with the extracts to study the synergistic effect. The element in 1830 by Sweedish physician and chemist Nils Gabriel Sefstrom, who named it vanadium [23]. Since then, various studies have been conducted using vanadium. Heyliger et al. had reported recovery of blood sugar to a normal level and activation of the cardio function in streptozotocin (STZ) diabetic rat, by injecting vanadium in drinks [13]. There have also been domestic reports of substitutional food and natural products that could induce postprandial hypoglycemic effect using various plant extracts for a-glucosidase inhibition. Although studies have been conducted on the postprandial hyperglycemic inhibitory effect of northern bamboo leaf, bean sprout extract, silkworm, Cordyceps militaris, and Smilax China extract [21, 24, 25], inhibitory activity of green tea or F20 were found to be superior compared to previously study results. Moreover, although there had been studies on individual

inhibitory report on each of the tea extract and vanadium, no known studies had been identified on the α -glucosidase inhibitory activity for its combination mixture. Thus, this study is considered to be useful in its scarcity.

The superior effect of non fermented tea on ACE inhibitory activity is suggested to be due to their high content of catechins, i.e., epigallocatechingallate (EGCG), while fermented tea does not contain like green tea but theaflavin and thearubigin. The catechins especially epigallocatechingallate showed strong inhibitory effects on ACE activity in vitro [26-28]. In a study report on ACE inhibition using 9 different types of herbs, such as lavender, rose, and pineapple sage, rose showed 133% inhibition, pineapple sage at 70%, and 10 \sim 30% for the rest, showing that the rose has a high inhibiting activity, although simple comparison cannot be conducted due to the extraction concentration differences [26]. In the ACE inhibitory activity research of Gui Valley potato extract, 87.6% inhibition was observed at 200 µg/mL concentration, showing slightly better inhibitory effect compared to green tea extract of the same concentration [27]. ACE, which is currently used as the anti-hypertensive activity index, belongs to the zinc metal protease group, covering the pulmonary hemangioendothelial. Composed of 10 peptides, Angiotensin I is metabolized into active angiotensin II for blood pressure elevation by ACE, after decomposition by renin. Active angiotensin II promotes aldosterone and anti-diuretic hormone (ADH) secretion, to reduce bood water loss and stimulate peripheral vascular contraction, to result in elevated blood pressure [29]. As one of the most representative adult disease, hypertension is a chronic degenerative disorder that causes circulatory disorders. With the increase of elderly population, its incidence rate has also increased. Thus, existence of natural biologically active substances in drinks and food we administer on a daily basis, is expected to be useful in a preventative medical point of view. As green tea and fermented tea have both been studied to show high ACE inhibitory activity as well as postprandial hypoglycemic effect, such products may be applied effectively for disorders such as hypertension and diabetes that require continuous control chemosynthetized enalapril and captopril have been developed and are being used as ACE inhibitors. However since such products have been known to show various physical side effects, development of natural anti-hypertensive products than chemical synthetics are in desperate need.

Functionality of tea is based on different active material of green tea and fermented tea, and the important biologically active material in fermented tea have been reported to be theaflavin and thearubigin, and not catechin [30]. According to study reports, anti-diabetic functions are different per its active materials, with green tea usually effective in fasting blood sugar disorder, where as fermented teas are effective in glucose tolerance disorder [30]. However, as only caffeine and EGCG was measured in this study, additional efficacy on tea is considered necessary as no measurement was conducted for the active contents of fermented tea such as theaflavin and thearubigin. Not much difference was observed in caffeine content in either green tea or fermented tea, although F50 showed an approximately 25% lower concentration compared to green tea. As an average adult caffeine administration is 400 mg/day and 200 mg/day in pregnant women [31]. 1L of tea drinking on a daily basis would not be much of a problem, as it does not exceed the recommended volume.

In conclusion, both green tea and fermented tea showed superior biological activity in this study, and correlation could be identified between high catechin content in green tea with its high α -glucosidase and ACE inhibition effects. Especially with the synergistic effect of the trace amount of green tea extract and vanadium combination compared to single green tea extract on α -glucosidase inhibition, such product is considered suitable as a preventative food to take for interactive disorder such as hypertension and diabetes of ascendant effects. As Jeju Island has a vast amount of underground water with high vanadium content compared to other regions, prophylaxis against diabetes and hypertension is considered possible when teas are made with vanadium containing underground water. Additional experimental and clinical studies for patients or healthy volunteers are needed in order to further elucidate on the exact mechanisms responsible for the postprandial hyperglycemia and ACE of green tea and fermented teas.

요 약

현대인의 기호식품인 차를 이용하여 비 발효차인 녹차와 3가 지 발효차로 구분하여 식후 고혈당의 지표가 되는 알파글루코시 데이스(α-glucosidase) 활성과 고혈압활성의 지표로 사용되는 안지오텐신전환효소(angiotensin converting enzyme, ACE)의 억제작용을 확인하였다. α-glucosidase 억제 활성의 상승효과 를 알아보기 위해 녹차추출물에 인슐린과 유사기능이 있다고 밝 혀진 바나듐을 미량(50 μg/mL)으로 혼합하여 α-glucosidase 활성 억제를 위한 상승효과 여부를 조사하였다. 또한 녹차의 기 능성물질로 알려진 에피갈로카테킨 갈레이트(epigallocatechin gallate, EGCG)의 농도와 카페인(caffeine)의 농도를 측정하 여 녹차와 발효차 효능을 분석하고자 하였다. 녹차와 발효차 추 출물로부터 식후 혈당을 증가시키는 α-glucosidase 활성이 크 게 억제되어 차의 식후 혈당저해 효과가 있음을 확인하였으며 차추출물 중에서도 20% 발효차가 가장 우수한 기능을 보였다. 그러나 차 추출물만을 단독으로 처리한 경우와 바나듐과 함께 처리한 경우 서로 다른 결과를 보였다. α-glucosidase 저해효 과는 저 농도(50 µg/mL)의 녹차와 바나듐 혼합 처리에서 단독 처리에 비해 저해활성이 2배나 증가되어 녹차가 바나듐과 가장 큰상승효과를 보임을 알 수 있었다. ACE 저해활성은 모든 차 추 출물에서 확인되었으나 녹차에서 가장 높게 조사되어 녹차가 다 른 차에 비해 가장 높게 측정된 EGCG 농도의 효과로 생각되며 발효차의 식후 혈당강하 및 ACE 저해효과는 EGCG 외에 또 다 른 기능성물질이 관여한 것임을 알 수 있었다.

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Author's information (Position): Park SY, Professor.

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