

Full Length Research Paper

Catechin-enriched green tea extract as a safe and effective agent for antimicrobial and anti-inflammatory treatment

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Accepted 9 September, 2011

The aim of this study was to examine the mechanism of green tea in treating a broad spectrum of human diseases and to develop a safe and effective agent for antimicrobial and anti-inflammatory application. Catechin-enriched green tea extract (CEGTE) was prepared containing $83.15 \pm 0.79\%$ of total catechins. *In vitro* antimicrobial and *in vivo* anti-inflammatory effects were investigated and safety was evaluated *in vivo* with relevant positive controls. CEGTE was found to have significant bacteriostatic effects in all the bacteria isolates tested and inhibitory effects on influenza virus A3 (H3N2) and coxsackie virus B4. CEGTE showed inhibitory effects both on carrageenin-induced paw edema in rats and xylene-induced ear edema in mice and exhibited an inhibitory response against acetic acid-induced writhing in mice. The oral LD₅₀ of CEGTE in both *Sprague Dawley* (SD) rats and imprinting control region (ICR) mice was determined to be greater than 20000 mg/kg body weight. The bacterial Ames test and mammalian micronucleus and sperm abnormality assay in mice showed no mutagenic properties with CEGTE. CEGTE can be applied as a safe and effective antimicrobial and anti-inflammatory agent with advantages over medicines which treat bacterial and viral infection or inflammation alone.

Key words: Catechin-enriched green tea extract (CEGTE), antimicrobial, anti-inflammatory, safety evaluation.

INTRODUCTION

Tea was originally used as folk medicine in China and later was drunk as a popular beverage worldwide. In recent years, special interest has been shown in its extensive health benefits ranging from antioxidant, anti-carcinogenic and antimicrobial effects to protective and even curative potency against cardiovascular diseases and cancers (Schneider and Segre, 2009). These properties have been mainly ascribed to its phenolic compounds, such as catechins. Green tea has the highest catechin content among all types of tea and considerable efforts have been directed towards understanding the cellular and molecular basis for these benefits (Yang et al., 2003).

As for antimicrobial properties, tea polyphenols have demonstrated potent activity against a broad range of harmful bacteria, such as *Staphylococcus aureus*

Streptococcus mutans, *Plesiomonas shigelloides*, *Salmonella typhi* and *Helicobacter pylori* (Sakanaka et al., 1989), including β -lactam *S. aureus* (Stapleton et al., 2004).

Pathogenic viruses and bacteria are causative infectious agents and probably linked to inflammatory abnormalities. An association between infection and inflammation has been suggested both in clinical and epidemiologic studies (Finlay and McFadden, 2006). Inflammatory abnormalities comprise a variety of unrelated human disorders and have been thought to be one of the main etiological origins of many chronic human diseases, including cancers (Grivennikov et al., 2010), atherosclerosis (Patel et al., 2008) and neurodegenerative diseases like Alzheimer's disease and Parkinson's disease (Glass et al., 2010). Antimicrobial and anti-inflammatory agents would obviously play a unique role in the prevention of a variety of human diseases. This study investigated the antimicrobial and anti-inflammatory effects of prepared catechin-enriched green tea extract (CEGTE) and assessed its safety aimed at understanding

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Table 1. MIC₅₀ of CEGTE for different bacteria (mg/ml).

Bacteria	Strains (n)	MIC ₅₀
<i>Staphylococcus aureus</i>	30	0.625 ± 0.041
<i>Streptococcus viridans</i>	10	2.50 ± 0.10
<i>Streptococcus pyogenes</i>	5	0.625 ± 0.032
<i>Pneumococcal bacteria</i>	10	1.25 ± 0.01
<i>Haemophilus influenzae</i>	4	1.25 ± 0.06
<i>Acinetobacter baumannii</i>	6	0.625 ± 0.038
<i>Klebsiella pneumoniae</i>	8	0.625 ± 0.003
<i>Proteus mirabilis</i>	7	1.25 ± 0.04
<i>Escherichia coli</i>	18	1.25 ± 0.02
<i>Salmonella typhi</i>	14	0.625 ± 0.017
<i>Enterobacter</i>	6	0.625 ± 0.011
<i>Enterococcus</i>	5	1.25 ± 0.08
<i>Pseudomonas aeruginosa</i>	8	10.0 ± 0.14
<i>Neisseria gonorrhoeae</i>	4	2.50 ± 0.11
<i>Neisseria meningitides</i>	7	5.0 ± 0.1
<i>Shigella flexneri</i>	7	0.625 ± 0.033
<i>Peptococcus</i>	8	2.50 ± 0.16
<i>Peptostreptococcus</i>	10	2.50 ± 0.07
<i>Lactobacillus</i>	7	2.50 ± 0.05
<i>Fusobacterium</i>	7	1.25 ± 0.03
<i>Bacteroides fragilis</i>	15	2.50 ± 0.12
<i>Bacteroides melaninogenicus</i>	10	2.50 ± 0.07
<i>Streptococcus mutans</i>	8	2.50 ± 0.10
<i>Propionibacterium shermanii</i>	7	2.50 ± 0.06
<i>Bifidobacterium bifidum</i>	6	2.50 ± 0.12

CEGTE, catechin-enriched green tea extract; MIC, minimum inhibitory concentration. The antibacterial activity was determined by standard doubling dilution method. Each MIC was determined from five independent experiments performed in duplicate.

the mechanism of green tea in the treatment of a broad spectrum of human diseases and at developing a safe and effective agent for such an application.

MATERIALS AND METHODS

CEGTE preparation and chemical analysis

Fresh shoots from the tea plant were inactivated by thermal steaming and oven dried at 80°C until fully dried. This dried green tea was then pulverized into 20 to 30 mesh particles and extracted with two changes of de-ionized water (1:10, w/v) at 90°C for 30 min. Both rounds of supernatants were collected and extracted using chloroform (1:1, v/v) three times at room temperature. The resultant water fraction was extracted three times using ethyl acetate (1:1, v/v). Collected ethyl acetate fraction was further extracted with de-ionized water (1:1, v/v) three times and the resultant ethyl acetate fraction was evaporated in vacuum at 40°C to a concentrated extract. The concentrated extract was suspended in de-ionized water and evaporated in vacuum at 40°C to recover ethyl acetate. Finally, the resultant concentration was freeze dried and CEGTE was obtained. Total polyphenol content was measured using the Folin-Ciocalteu colorimetric method and catechins and caffeine were analyzed by high performance liquid chromatography

(Nishitani and Sagesaka, 2004). The level of polyphenolic compounds was confirmed at 98 ± 3.52% in the prepared CEGTE, containing 83.15 ± 0.79% of the total catechins, comprising 0.22 ± 0.01% catechin (C), 0.79 ± 0.03% epicatechin (EC), 0.38 ± 0.01% catechin gallate (CG), 17.28 ± 0.59% epicatechin gallate (ECG), 0.31 ± 0.01% gallocatechin (GC), 0.92 ± 0.02% epigallocatechin (EGC), 2.44 ± 0.07% gallocatechin gallate (GCG), 60.94 ± 2.31% epigallocatechin gallate (EGCG) and 0.069 ± 0.001% caffeine, respectively.

Clinical isolates of bacteria and viruses

Clinical isolates of bacterial strains (Table 1) were obtained from the Children's Hospital, the 1st and 2nd Affiliated Hospitals of Zhejiang University, Zhejiang People's Hospital, 1st Hangzhou People's Hospital and the 9th Shanghai People's Hospital in China. Influenza virus A3 (H3N2) was obtained from the Virology Institute, Chinese Academy of Preventive Medicine, Beijing and coxsackie virus B4 was from the Wuhan Institute of Virology, Chinese Academy of Sciences.

Animals and husbandry

Sprague Dawley (SD) rats and imprinting control region (ICR) mice were obtained from the Laboratory Animal Center of Zhejiang

Province, China (License No: SCXK (Zhe) 2008-0033). The animals were housed in standard laboratory conditions ($22 \pm 1^\circ\text{C}$, $50 \pm 10\%$ humidity and a 12 h light/dark cycle) and supplied with commercial feed (Suzhou Shuangshi Laboratory Animal Feed Co., Jiangsu, China) and water *ad libitum*. Animal care was in accordance with the principles of Laboratory Animal Care and Use in Research (Ministry of Health, Beijing, China).

Antibacterial activity

Antibacterial activity was determined using the standard doubling dilution method. The CEGTE was diluted with distilled water and subjected to a doubling dilution series with the final solutions of 80, 40, 20, 10, 5.0, 2.5, 1.25 and 0.625 mg/ml in test tubes or plate wells containing medium Mueller-Hinton or nutrition broth with serum inoculated with different test bacterial suspensions at a final concentration of 1×10^6 CFU/ml. The plates or tubes were incubated at 37°C for 18 h for aerobic strains and at 37°C for 48 h for anaerobic bacteria. Microbial growth was observed macroscopically and minimal inhibitory concentration (MIC) was defined as the lowest CEGTE concentration at which no growth was observed after incubation. Each MIC was determined from five independent experiments performed in duplicate.

Antiviral activity determination

The effects of CEGTE on influenza virus A3 (H3N2) were determined using the hemadsorption test. Madin-Darby canine kidney (MDCK) cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum on 96-well plates (Corning Costar Corp., New York, USA) in 5% CO_2 at 37°C until confluent monolayer cells were formed and were then transferred into serum-free DMEM. After being cultured overnight, the MDCK cells were washed three times with serum-free DMEM and incubated with influenza virus A3 (H3N2) (30 HAF/0.1 ml) in the presence of different CEGTE or drug concentrations for 1 h at 33°C . The MDCK cells were washed twice with serum-free DMEM. Cultured in serum-free DMEM (150 μl /well) supplemented with 2 $\mu\text{g}/\text{ml}$ trypsin (Difco, Michigan, USA) for 20 h, MDCK cells were added to 2.5% chicken erythrocyte suspension (30 μl /well) and incubated at room temperature for 1 h. Unbound erythrocytes and medium were gently removed by inverting the plates for 5 min. The wells were monitored microscopically ($\times 60$) for hemadsorption quantification and over 10 erythrocytes adsorbed in a single cluster was counted as a Hemadsorbing foci (HAF).

CEGTE effects on coxsackie virus B4 were investigated using a plaque inhibition assay. Vero E6 cells were seeded onto 24-well plates (Corning Costar Corp., New York, USA) and cultured in 5% CO_2 at 37°C until confluent monolayer cells were formed. The cell monolayer was washed with serum-free DMEM three times, and then infected with coxsackie virus B4 (60 PFU/0.3 ml) in the presence of different CEGTE or drug concentrations at 37°C for 1 h. The Vero cells were washed twice with serum-free DMEM and were cultured further in 1% agarose DMEM (0.5 ml/well) for 72 h. The Vero cells were added to 2% crystal violet (0.5 ml/well) and incubated at room temperature for 3 h. Finally the cells were washed with water to remove the agarose, and plaque numbers were determined visually over a light box.

Carrageenin-induced rat paw edema

SD rats (136.8 ± 5.9 g) were randomly divided into five groups ($n = 10$). Saline (negative control), CEGTE (75, 150 or 300 mg/kg body weight [BW], treatment) or aspirin (200 mg/kg BW, positive control) were given through stomach perfusion once daily for 3 days.

Carrageenin (0.1 ml from a 10 mg/ml solution) was injected into the plantar aponeurosis of the right hind paw in rats of all five groups 1 h after the last administration of saline, CEGTE or aspirin. The paw volume was measured at 0, 1, 2, 3, 4, 5 and 6 h, respectively following the carrageenin injection and the ratio of the volume increment to the initial volume was recorded as the paw edema rate.

Xylene-induced mice ear edema

ICR mice (24.6 ± 0.9 g) were distributed randomly in five groups ($n = 10$). Saline (negative control), CEGTE (75, 150 or 300 mg/kg BW, treatment) or aspirin (200 mg/kg BW, positive control) were administered by stomach perfusion once daily for 3 days. The inner surface of the right ear of the mice was touched by xylene for 5 s, 1 h after the last stomach perfusion. The mice were sacrificed by cervical dislocation 15 min after the touch and punches were made in the ear using an 8 mm cork borer. Each ear disc was weighed and right and left ears were compared. The difference between the right and left ears due to xylene was recorded as the measure of ear edema.

Acetic acid-induced mice writhing

ICR mice (23.2 ± 1.2 g) were randomized into five groups ($n = 10$) and were given saline (negative control), CEGTE (75, 150 or 300 mg/kg BW, treatment) or aspirin (200 mg/kg BW, positive control), respectively through stomach perfusion once daily for 3 days. 1 h after the last stomach perfusion, each animal was injected with 0.7% acetic acid (0.1 ml/10 g of mice, intraperitoneally) and the number of writhing movements was recorded within a period of 15 min and the latent time was observed.

Acute toxicity in rats and mice

SD rats (180 to 220 g) and ICR mice (18 to 22 g), 10 males and 10 females of both animals, were fasted overnight but given water *ad libitum* prior to dosage. CEGTE (500 mg/ml distilled water, as the maximum tolerant dose for gavage) was administered by oral gavage at a dose of 20000 mg/kg BW twice with a 4 h interval in both rats and mice. Observation of the general status, symptoms of toxicity and mortality in rats and mice was continued for two weeks.

Ames test

The Ames test was conducted with four histidine-requiring *Salmonella typhimurium* (*S. typhimurium*) mutant strains TA97, TA98, TA100 and TA102 obtained from the Shanghai Municipal Center for Disease Control & Prevention, China, using the treat and plate method. Tester bacteria were exposed to four CEGTE concentrations ranging from 8 to 1000 $\mu\text{g}/\text{plate}$, with or without S_9 mixture, respectively. Three parallel plates were tested for each concentration. Negative and positive controls were run simultaneously with the test. All the tests were performed twice.

Micronucleus assay in mice

ICR mice (25 to 30 g) were randomized into five groups, each with five males and five females. CEGTE (125, 250 and 500 mg/ml distilled water) was administered by oral gavage (20 ml/kg BW) twice with a 24 h interval to three animal groups, respectively, with corresponding doses of 2500, 5000 and 10000 mg/kg BW. Distilled water and cyclophosphamide (60 mg/kg BW) were given in a similar

way to the remaining mice as negative and positive controls. The animals were killed 6 h after the final administration and their sternum marrow cells were analyzed following methanol fixing and Giemsa staining. The frequency of micronuclei was counted based on an examination of 1000 polychromatic erythrocytes (PCE) per mouse.

Sperm abnormality test in mice

Male ICR mice (25 to 30 g) were randomized into five groups, each with seven animals. CEGTE was given by oral gavage once daily for 5 days to three animal groups in the same concentration, volume and doses as in a micronucleus assay. Distilled water and mitomycin C (2.0 mg/kg BW) were given in a similar way to the remaining mice as negative and positive controls. The animals were killed by cervical dislocation 35 days after the first administration. The bilateral epididymides of five animals chosen randomly from the seven were placed in physiological saline and minced with ophthalmic scissors. Smears were prepared on clean slides, air-dried, fixed with methanol and stained with 1% eosin. Morphological observation on sperm was carried out microscopically ($\times 100$). For each animal 1000 sperm were observed to assess morphological abnormalities, including amorphous, hookless, banana-shaped and tail abnormalities.

Statistical analysis

The results were expressed as the means \pm SD and evaluated by analysis of variance (ANOVA) followed by Tukey's studentized range test carried out on the Statistical analysis system (SAS) for windows V9, and $P < 0.05$ was regarded as statistically significant.

RESULTS

Antibacterial effects

The results from the standard doubling dilution method (Table 1) by MIC_{50} values showed that CEGTE exhibited strong bacteriostatic and bactericidal activity against all the tested bacterial isolates with slightly weaker against *Pseudomonas aeruginosa* ($MIC_{50} = 10$ mg/ml) followed by *Neisseria meningitides* ($MIC_{50} = 5$ mg/ml). MIC_{50} for the other bacterial isolates were between 0.625 to 2.5 mg/ml. The most notable antibacterial effects were demonstrated against aerobic and anaerobic strains often found in the human oral cavity. CEGTE showed a greater antibiological potency against *S. aureus* ($MIC_{50} = 0.625$ mg/ml) than against *Streptococcus viridans* ($MIC_{50} = 2.5$ mg/ml).

Antiviral activity

The inhibitory activities of both CEGTE and the positive control drug ribavirin on influenza virus A3 (H3N2) as shown by the hemadsorption test were presented in Figure 1A. Abundant HAF were observed in the negative control where only the virus was seeded, while HAF decreased in the positive control where the drug was added. CEGTE exhibited strong inhibitory activity on the

hemadsorption ability of influenza virus A3 (H3N2) with an IC_{50} of 4.89 μ g/ml, slightly greater than that of ribavirin ($IC_{50} = 3.85$ μ g/ml).

The antiviral effects of CEGTE and of the positive control drug ribavirin as demonstrated by the plaque inhibition assay at different concentrations were shown in Figure 1B. CEGTE inhibited the plaque-forming activity of coxsackie virus B4 in a dose-dependent manner with an IC_{50} of 5.85 μ g/ml, approximately twice that of ribavirin ($IC_{50} = 2.58$ μ g/ml).

Effects on carrageenin-induced rat paw edema

Carrageenin-induced paw edema in rats increased gradually with time following the injection. CEGTE inhibited carrageenin-induced paw edema in a dosage-dependent manner. As shown in Figure 2, CEGTE significantly inhibited carrageenin-induced paw edema at a high dose as measured at all hourly time points ($P < 0.01$) to an extent similar to aspirin except for at 1 and 4 h ($P < 0.05$). Medium dose CEGTE showed inhibitory activity at 2 and 4 h after the induction of paw edema ($P < 0.05$), while inhibition in low dosage CEGTE reached significant difference at 5 h ($P < 0.05$) as compared with the negative saline control.

Effects on xylene-induced mice ear edema

A similar inhibitory activity pattern was observed in xylene-induced mice ear edema. Compared with the negative control, a remarkable inhibition of xylene-induced mice ear edema was demonstrated by CEGTE in all the low, medium and high dosages ($P < 0.01$) (Figure 3), although, slightly less than the inhibition shown by the positive control aspirin.

Effects on acetic acid-induced mice writhing response

CEGTE exhibited an inhibitory response against acetic acid-induced writhing in mice. The number of writhing movements was reduced in CEGTE groups at all dosages as compared to the animals receiving saline. However, reduction in a number of writing movements only reached significant difference level ($P < 0.01$) with high and medium dosage CEGTE as shown in Figure 4A while the smaller dosages did not result in statistical significance. Figure 4B showed that the latent time in writhing response was prolonged significantly in the high dosage CEGTE ($P < 0.01$) and aspirin ($P < 0.01$) groups as compared to the negative control, while with low and medium dosage CEGTE latent time was only slightly shorter than in the saline group. The positive control aspirin demonstrated greater inhibition of the number of writhing movements and a more prolonged latent time

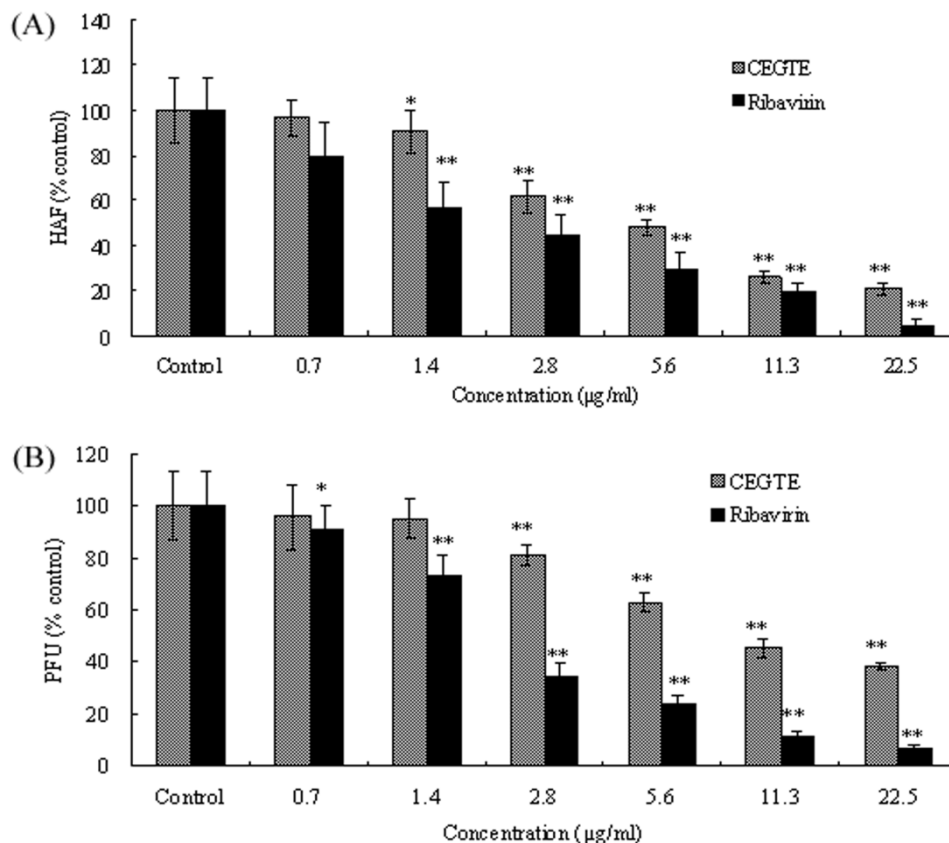


Figure 1. Inhibitory effects of CEGTE on (A) Influenza virus A hemadsorption and (B) Coxsackie virus B4 plaque formation. The method used for Influenza virus A3 (H3N2) was the hemadsorption test and for Coxsackie virus B4 a plaque inhibition assay. Over 10 erythrocytes adsorbed in a single cluster were counted as a HAF. Ribavirin was used as the positive control and distilled water as the negative control. Inhibition of hemadsorption was observed both by CEGTE ($IC_{50} = 4.89 \mu\text{g/ml}$) and ribavirin ($IC_{50} = 3.85 \mu\text{g/ml}$) and plaque formation inhibition by CEGTE ($IC_{50} = 5.85 \mu\text{g/ml}$) and ribavirin ($IC_{50} = 2.58 \mu\text{g/ml}$). Statistical significance * $P < 0.05$ and ** $P < 0.01$ versus the negative control. CEGTE, catechin-enriched green tea extract; PFU, plaque-forming unit.

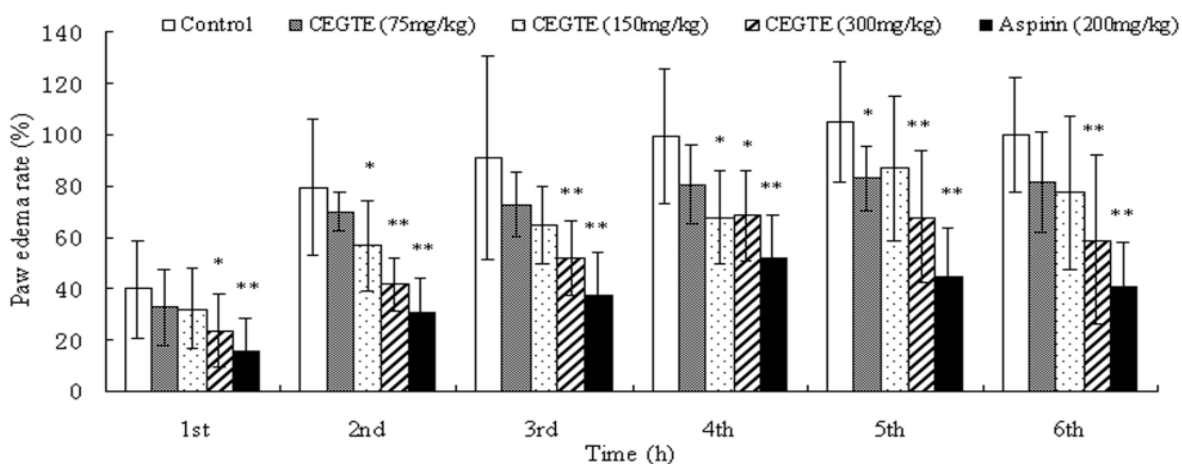


Figure 2. Effects of CEGTE on carrageenin induced rat paw edema. Aspirin was used as the positive control and distilled water as the negative control. CEGTE, aspirin and distilled water were all given by stomach perfusion. The ratio of the volume increment to the initial volume was defined as the paw edema rate. Statistical significance * $P < 0.05$ and ** $P < 0.01$ versus the negative control. CEGTE, catechin-enriched green tea extract.

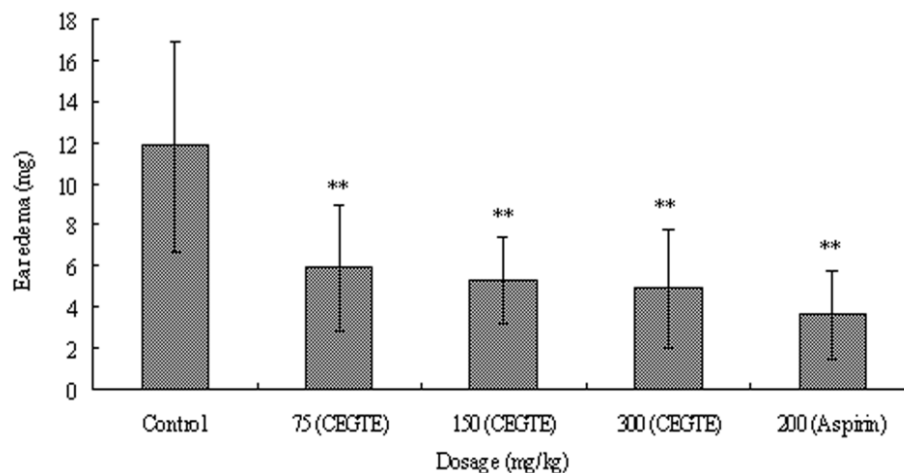


Figure 3. Effects of CEGTE on xylene-induced mice ear edema. Aspirin was used as the positive control and distilled water as the negative control. CEGTE, aspirin and distilled water were all given by stomach perfusion. The difference in weight between the right and left ears due to xylene application was obtained as the measure of ear edema. Statistical significance * $P < 0.05$ and ** $P < 0.01$ versus the negative control. CEGTE, catechin-enriched green tea extract.

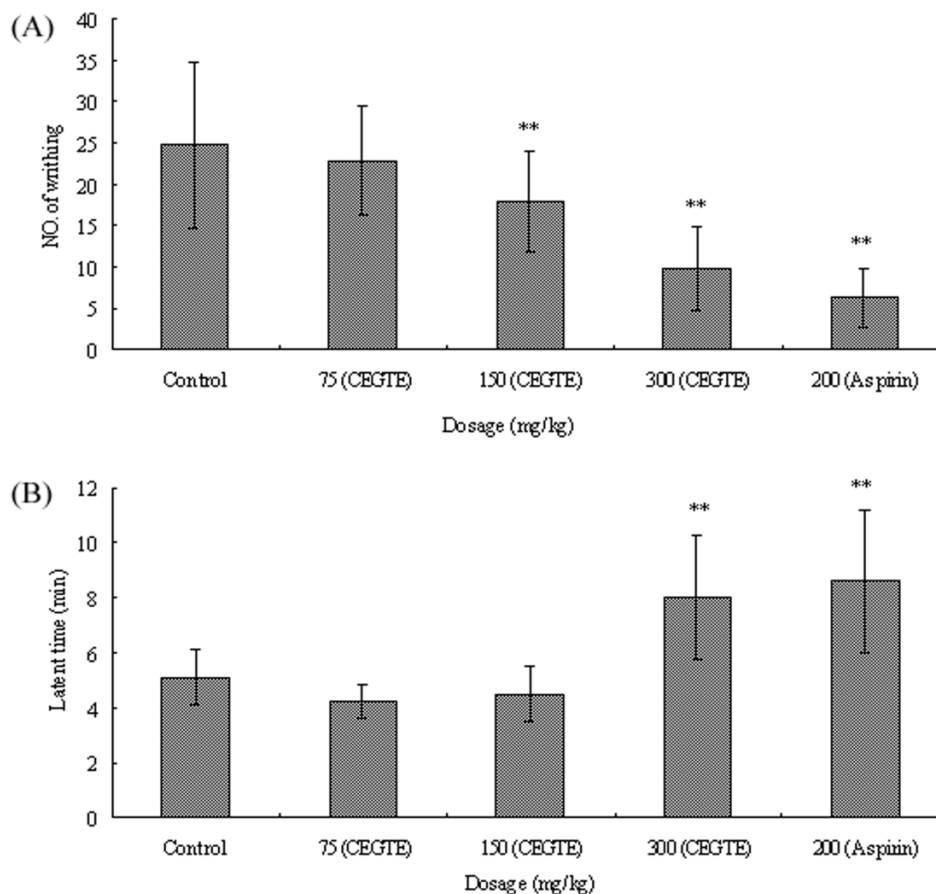


Figure 4. Effects of CEGTE on the mice response to acetic acid induced writhing. Aspirin was used as the positive control and distilled water as the negative control. CEGTE, aspirin and distilled water were all given by stomach perfusion. Writhing number was counted within a period of 15 min (A) and latent time was recorded (B). Statistical significance * $P < 0.05$ and ** $P < 0.01$ versus the negative control. CEGTE, catechin-enriched green tea extract.

than CEGTE.

Oral acute toxicity and mutagenicity risk

Throughout the 14-day acute toxicity study, no death occurred either in rats or mice administered with CEGTE (20000 mg/kg BW). Both male and female animals showed steady increase in body weight and exhibited no abnormalities in their external appearance or behavior. The body weight increased from 191.56 ± 6.5 g to 274.4 ± 9.7 g for female rats and from 198.9 ± 8.2 g to 332.3 ± 14.8 g in male rats during the 14 days period, while female mice increased from 18.7 ± 0.6 g to 29.6 ± 1.4 g and male mice from 19.2 ± 0.6 g to 34.3 ± 1.8 g. Macroscopic observation of both rats and mice at necropsy did not show any alteration in the shape or color of external surfaces nor in any orifices of the cranial, thoracic and abdominal cavities and lymphatic nodes. Oral LD₅₀ of CEGTE in both SD rats and ICR mice was determined to be greater than 20000 mg/kg BW.

No remarkable increase in the number of revertant colonies occurred in the four test strains with any CEGTE concentration against the negative or solvent controls either with or without S₉ mix. Similar results were obtained from the two duplicate tests (Table 2). No obvious dose-response relationship was observed and the number of revertant colonies was not found to be more than double those of the negative and solvent controls. Therefore, the Ames test result for CEGTE was considered to be negative.

The results of micronucleus assay in mice were given in Table 3. No significant difference was observed between the three treatment groups and the negative control for the number of micronucleated PCEs or the incidence of micronucleated PCEs, while a significant increase ($P < 0.01$) was demonstrated for both micronucleated PCE counts and relevant incidence in the positive control mice receiving cyclophosphamide. Therefore, the results of micronucleus assay in mice for CEGTE were also considered to be negative.

As shown in Table 4, the frequency of sperm abnormalities ranged from 2.44 to 2.84% for the three CEGTE groups and was 2.32% for the negative control. No significant difference was observed between the CEGTE groups and the negative control ($P > 0.05$). However, the frequency of sperm abnormalities in the positive control Mitomycin C mice was 5.44%, producing a statistically significant elevation of abnormal sperm as compared to the negative control ($P < 0.05$).

DISCUSSION

In this study, CEGTE demonstrated strong antibacterial activity against a variety of clinical bacteria isolates (Table 1) and powerful antiviral potency against both

Influenza virus A3 (H3N2) and Coxsackie virus B4 (Figure 1). These were in agreement with outcomes found earlier both in a broad range of bacteria (Bandyopadhyay et al., 2005) and virus (Nakayama et al., 1993) tests with tea extract or tea catechins. CEGTE was also demonstrated to possess potent anti-inflammatory effects in animal models of carrageenin-induced rat paw edema (Figure 2), xylene-induced mice ear edema (Figure 3) and acetic acid-induced mice writhing (Figure 4). Tea catechins have extensively been found to be capable of treating inflammatory disorders in animal models. Different tea products attenuated inflammation in *Trypanosoma brucei* infected mice (Karori et al., 2008). EGCG protected toluene diisocyanate-induced airway inflammation in a murine asthma model (Kim et al., 2006), and decreased the risk of cardiovascular diseases by reducing inflammatory markers in rats fed with an atherogenic diet (Ramesh et al., 2010). Histopathological experiments found green tea extract to significantly reduce paraquat-induced pulmonary edema and hemorrhage in rats (El-Sayed et al., 2009). In human subjects, an association between habitual tea consumption and markers of chronic inflammation was demonstrated in an epidemiological investigation (Bacquer et al., 2006). Pharyngitis is one of the common diseases characterized by the typical signs of inflammation signs: rubor, tumor, calor and dolor. In our clinical trial, green tea polyphenols were found as an effective phytochemical in the treatment of pharyngitis (Wang et al., 2011). Furthermore, in the present study, CEGTE was shown to be practically non-toxic in the bacterial and animal experiments. Oral LD₅₀ of CEGTE was determined to be greater than 20000 mg/kg BW in both SD rats and ICR mice. Oral gavage of CEGTE at concentrations up to 10000 mg/kg BW did not cause any mortality or treatment-related clinical signs and did not result in a significant increase in the incidence of micronucleated PCEs or the frequency of sperm abnormalities in mice. Thus, it is suggested that CEGTE could be applied as a safe and effective antimicrobial and anti-inflammatory agent.

Antimicrobial agents are important clinical tools for dealing with infection. However, concern often arises about the adverse effects of such treatment (Sanderson et al., 2004). Antibiotic-associated diarrhea incidence varies from 5 to 25% (Bergogne-Berezin, 2000). The genotoxic potential of metronidazole has been observed, with the frequencies of micronucleated PCEs increasing in *Oreochromis niloticus* (Çavas and Ergene-Gözükara, 2005). Ribavirin, used as the positive control treatment in the present study, has been observed to induce sperm abnormalities in rats (Narayana et al., 2002). However, the safety of tea has long been demonstrated, since the consumption of tea has a long history. Furthermore, the present study confirmed that CEGTE produced no toxic risk or adverse effects in the safety experiment with rats or mice.

Table 2. Ames test results for CEGTE in four strains of *S. typhimurium*.

Strain	Dose level ($\mu\text{g}/\text{plate}$)	Revertant colonies			
		-S9		+S9	
		Test 1	Test 2	Test 1	Test 2
TA97	0	121 \pm 2.6	126 \pm 4.5	138 \pm 2.6	139 \pm 3.1
	8	113 \pm 3.6	124 \pm 2.6	133 \pm 1.5	129 \pm 3.5
	40	121 \pm 2.6	127 \pm 3.1	138 \pm 3.1	139 \pm 2.6
	200	134 \pm 2.1	132 \pm 2.1	153 \pm 3.2	149 \pm 5.5
	1000	145 \pm 4.6	139 \pm 4.4	163 \pm 5.6	158 \pm 4.0
	DMSO	123 \pm 5.9	122 \pm 3.8	139 \pm 7.2	138 \pm 4.0
	ICR-191(1.0)	3012 \pm 99.0	2880 \pm 166.2		
	2-AF (10.0)			1923 \pm 164.2	1857 \pm 194.7
TA98	0	34 \pm 2.1	33 \pm 0.6	39 \pm 1.0	39 \pm 1.5
	8	32 \pm 1.0	32 \pm 1.0	34 \pm 1.0	34 \pm 1.5
	40	34 \pm 1.5	33 \pm 1.0	37 \pm 1.5	38 \pm 1.0
	200	36 \pm 2.5	36 \pm 0.6	40 \pm 1.2	40 \pm 1.0
	1000	40 \pm 2.6	38 \pm 1.5	41 \pm 1.2	43 \pm 1.2
	DMSO	36 \pm 1.2	33 \pm 1.5	41 \pm 1.5	41 \pm 1.0
	DNR (6.0)	2196 \pm 162.1	1951 \pm 128.5		
	2-AF (10.0)			2351 \pm 173.9	1940 \pm 72.3
TA100	0	128 \pm 3.0	128 \pm 2.1	144 \pm 4.5	142 \pm 3.1
	8	126 \pm 4.5	126 \pm 5.3	128 \pm 4.0	133 \pm 2.6
	40	126 \pm 5.0	123 \pm 1.0	139 \pm 4.4	132 \pm 4.6
	200	126 \pm 4.7	127 \pm 3.2	146 \pm 8.1	140 \pm 1.5
	1000	130 \pm 6.5	132 \pm 4.7	150 \pm 1.5	149 \pm 4.9
	DMSO	131 \pm 3.2	133 \pm 4.2	140 \pm 2.6	140 \pm 2.5
	NaN ₃ (1.5)	2115 \pm 119.2	1965 \pm 217.8		
	2-AF(10.0)			1822 \pm 125.2	1912 \pm 187.1
TA102	0	266 \pm 9.7	266 \pm 6.5	274 \pm 5.0	275 \pm 6.0
	8	257 \pm 13.1	255 \pm 4.7	255 \pm 9.1	257 \pm 3.6
	40	258 \pm 9.0	259 \pm 6.2	259 \pm 11.2	268 \pm 3.5
	200	273 \pm 10.8	266 \pm 7.0	277 \pm 9.8	283 \pm 7.2
	1000	282 \pm 6.8	273 \pm 3.2	296 \pm 10.0	288 \pm 14.0
	DMSO	268 \pm 7.0	265 \pm 5.1	277 \pm 3.5	274 \pm 5.6
	MMC (0.5)	1848 \pm 108.6	1819 \pm 97.7		
	1,8-DAA (50.0)			1849 \pm 79.1	1819 \pm 151.0

CEGTE, catechin-enriched green tea extract; DMSO, dimethyl sulfoxide; ICR-191, 6-Chloro-9-[3-(2-chloroethylamino)propylamino]-2-methoxyacridine dihydrochloride, an acridine mutagen; 2-AF, 2-aminofluorene; DNR, daunorubicin; MMC, mitomycin C; 1,8-DAA, 1,8-dihydroxyanthraquinone.

Resistance is another problem with antibiotics. *H. pylori* is one of the most common bacterial infections in humans and *H. pylori* resistance to metronidazole has been estimated to be 60 to 70% where antibiotic use is high. Components of green tea have been found to be effective in treating *Helicobacter*-induced gastritis in humans (Stoicov et al., 2009). *S. aureus* accounts for a great percentage of pharyngitis cases and tea has shown strong antibacterial activity against methicillin-resistant *S.*

aureus (MRSA) in elderly patients (Yamada et al., 2003). These findings suggest that CEGTE could be used together with antibiotics to strengthen their potency and thus, prevent antibiotic overuse. These are clear advantages of CEGTE use as an antimicrobial and anti-inflammatory agent.

An increasing number of studies suggest that persistent inflammation plays a critical role in the initiation and development of various diseases (Yu and Chung, 2006).

Table 3. Result of mice micronucleus test for CEGTE.

Treatment (mg/kg)	Mice number	PCE number	MNPCE number	MN (%)
Males				
0	5	5000	6	0.12 ± 0.08
2500	5	5000	9	0.18 ± 0.04
5000	5	5000	8	0.16 ± 0.11
10000	5	5000	8	0.16 ± 0.11
CPA	5	5000	92	1.84 ± 0.36**
Females				
0	5	5000	7	0.14 ± 0.11
2500	5	5000	7	0.14 ± 0.09
5000	5	5000	8	0.16 ± 0.05
10000	5	5000	7	0.14 ± 0.13
CPA	5	5000	101	2.02 ± 0.60**

CEGTE, catechin-enriched green tea extract; PCE, polychromatic erythrocytes; MNPCE, micronucleated PCE; MN, frequencies of micronucleus; CPA, cyclophosphamide. CPA was given to mice as the positive control at dose of 60 mg/kg by a single intraperitoneal administration. **CPA gave a statistically significant elevation of MN as compared to the negative control ($P < 0.01$). Distilled water was treated as the negative control.

Table 4. Result of mice sperm abnormality test for CEGTE.

Treatment (mg/kg)	Mice number	Sperm number	Abnormality number	SA (%)
0	5	5000	116	2.32 ± 0.23
2.5	5	5000	123	2.46 ± 0.17
5.0	5	5000	122	2.44 ± 0.43
10.0	5	5000	142	2.84 ± 0.56
MMC	5	5000	272	5.44 ± 1.14*

CEGTE, catechin-enriched green tea extract; SA, frequencies of sperm abnormalities; MMC, mitomycin C; MMC was given to mice as the positive control at dose of 2.0 mg/kg by a single intraperitoneal administration. Distilled water was treated as the negative control. *The positive control gave a statistically significant elevation of sperm abnormality frequencies as compared to the negative control ($P < 0.05$).

Interestingly, both epidemiological investigations and laboratory experiments show that tea exerts protective effects against a similar broad spectrum of unrelated diseases associated with chronic inflammation (Yang et al., 2003; Schneider and Segre, 2009). This similarity suggests that tea catechins are of clinical value in treating a variety of human diseases by blocking inflammatory progress. Tea catechins exert their anti-inflammatory effects through several different pathways. Antimicrobial effects serve as one of the underlying mechanisms. The present study confirms both the antimicrobial effects and anti-inflammatory potency of CEGTE. *Helicobacter* is one of the most common bacterial infectious agents. Green tea extract has been shown to prevent gastric mucosal inflammation when ingested prior to exposure to *Helicobacter* infection in mice (Stoicov et al., 2009) and reduce gastric epithelial cell proliferation and apoptosis stimulated by *H. pylori* infection in mice (Akai et al., 2007). Inflammation and disease are linked through the production of inflammatory

mediators. The induction of pro-inflammatory genes by tumor necrosis factor (TNF) has been linked to most diseases (Kundu and Surh, 2008). When exposed to TNF, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is activated in most cell types, leading to the expression of inflammatory genes such as cyclooxygenase-2 (COX-2), inflammatory cytokines and inducible nitric oxide synthase (iNOS). Tea polyphenols were found to inhibit COX-2 and iNOS expression by blocking NF- κ B activation (Kundu and Surh, 2008). ECG, EGC and EGCG have also been found to enhance the production of anti-inflammatory cytokine IL-10 (Crouvezier et al., 2001). Meanwhile, oxidant stress has been found to be another cause of inflammation (Sarkar and Fisher, 2006). Tea catechins have been recognized as a powerful antioxidant (Yang et al., 2009), and thus play a special role in its anti-inflammatory effects. Another of the anti-inflammatory mechanisms of tea catechins is to serve as a COX-2 inhibitor, similar to aspirin as the positive control in the present study.

However, aspirin resistance has been found in cardiovascular disease (Wong et al., 2004) and aspirin-induced asthma is of concern (Szczeplik and Stevenson, 2003). Tea catechins, on the other hand, increase the expression of anti-asthmatic markers and protect toluene diisocyanate-induced airway inflammation in a murine asthma model (Kim et al., 2006). The antioxidant capacity of tea catechins endows CEGTE with more clinical potential than that of sole anti-inflammatory agents.

Conclusion

CEGTE demonstrated potent antimicrobial and anti-inflammatory effects and represents a potential treatment for a broad spectrum of human diseases. CEGTE can be applied as a safe and effective antimicrobial and anti-inflammatory agent with many advantages. Carotid plaques have been found less frequently with increasing tea consumption in women (Debette et al., 2008). However, several studies have shown no effect of regular tea ingestion on circulating C-reactive protein concentration (Lee et al., 2005). Such inconsistency may at least partially, be due to a difference in physiological tea catechin concentration depending on the level of tea consumption. In this sense, CEGTE may be advantageous as an antimicrobial and anti-inflammatory agent, since adequate dosage would be available if given in CEGTE form.

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