Effect of Cu^{2+}on Antioxidant of EGCG

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Abstract— (−)-Epigallocatechin-3-gallate (EGCG), a major compound of tea polyphenols it has exhibited an antioxidant activity in previous studies. Cu2+ can be a center ion in complex reaction with EGCG and affect its antioxidant capacity as well. To investigate the effects on antioxidation of EGCG after add Cu2+ the pyrogallic acid is used as a reagent to test the oxidation which was added in liquid with different concentration gradent and proportions of Cu2+and EGCG. The result showed no significant（P=0.95） effects when concentration of EGCG and Cu2+ are 5μmol·L-1 and 10μmol·L-1, respectively. However, Cu2+ decreased the antioxidant activity of EGCG at 50μmol·L-1 while increased the antioxidant capacity at 100μmol·L-1. Cu2+ has no obvious effect on antioxidant capacity of EGCG before 50 minutes but improved the antioxidant of EGCG thereafter at 250μmol·L-1 while inhabited the antioxidant of EGCG in first 90 minutes at 500μmol·L-1 and improved the antioxidant thereafter. Different concentrations of EGCG have a greate difference in antioxidant property, it present obvious concentration-dependent. Furthermore, the data of antixidant dynamics of the different test system are also given in the study.

Keywords- EGCG; antioxidant; Cu2+ ; concentration-dependent

I. INTRODUCTION

Copper (Cu) is an important metal which belongs to transition metal elements in the periodic table. It is also an important part of ceruloplasmin in vivo, to participate in redox reaction. Meanwhile, it plays an important role for iron absorption and hemoglobin synthesis,[1] and can form a variety of hybrid orbital track, and be used as central ion accept multiple ligands form complexes. Especially, copper ions have a higher redox potential (Cu2 +/ Cu + =0.17v, Cu2 + / Cu = 0.34v) to oxidize (−) epigallocatechin-3-gallate (EGCG). [2]

Tea, Camellia sinensis (L.) Kuntze (Theacae), are one of the most widely consumed beverages in the world, and their main active compounds are catechins, including (−) epigallocatechin- 3-gallate (EGCG), (−) epigallocatechin (EGC) and (−) epicatechin gallate (ECG) [3] it is believed that possesses beneficial health effects. These benefits are attributed to the polyphenol EGCG, which is a major component of green tea, accounting for more than 4% of its dry weight and 50% to 60% of total catechins.[4,5-7] Tea catechins are primarily absorbed in the small intestine then are transported to other organs for instance, the brain[8]. Owning their unique chemical structure, catechins have varied pharmacological properties such as sedative, hypotensive, lipid-lowering and antitumori genesis effects [9-11]. Besides being able to scavenge reactive radicals and reduce peroxidative status [12-14], EGCG has been suggested to have neuro-protective effects to treat behavioral impairments induced by ischemia, toxins, stress, and hypertension [15-17]. Green tea catechins, especially EGCG, have potent antioxidative effects and is able to protect against various oxidative injuries [18]. EGCG has a protective role against reserpine-induced orofacial dyskinesia (OD), probably via its powerful antioxidative properties. Thus, EGCG may possible have a clinically relevant therapeutic effect in preventing, delaying or even treating tardive dyskinesia (TD) [19]. Recent research has suggested that EGCG possesses anti-tumor properties [20-22] mediated through inhibition of angiogenesis [22], tumorigenesis-related kinases [23], and other factors [24-27].

The tea catechins have been shown to possess antibacterial or bactericidal activities [28-32]. EGCG is a naturally broad spectrum antimicrobial agent. Bacterial species has been described included Escherichia coli, Stenotrophomonas maltophilia, Bacillus bacteria, Listeria monocytogenes, and Staphylococcus aureus [33-37]. Besides some study present EGCG can weaken toxicity of Cu2+ for the bacterial cells [33-34]. However, the easy oxidation of EGCG has limited its application. To increase the antimicrobial activity and stability of EGCG, the EGCG-Cu (II) complex was formed by chelating copper ions and then electronspun into polyvinyl alcohol (PVA) nanofiber in Sun’s study[38].

Copper ion concentrations in the organism are generally small amount, and the amount of EGCG people daily intake by drinking tea is also low. It is very interesting to study the interaction (oxidation and antioxidantion) between EGCG and copper ions at low concentration level (μmol /L). Recent studies have already demonstrated that pH value and the ratio of EGCG and to Cu2+ were main influence factors.[39].The first aim of this work was to test the effects of EGCG concentration and copper ions to antioxidation of EGCG. The second major aim was to address the question what is the effect to antioxidant of ratio between EGCG and Cu2+ in a low concentration level.

II. MATERIALS AND METHODS

A. *Materials*

(−)-Epigallocatechin-3-gallate (EGCG) (> 98%); Pyrogallic Acid(>99%) (1,2,3-Trihydroxybenene , CAS:

87-66-1) was purchased from Biopurify Phytochemicals Inc (Chengdu, China);Copper(II) sulfate(>99)(CAS: 7758-98-7) was purchased from Sinopharm Chemical Reagent Co.,Ltd .

B. Methods

The antioxidant capacity of EGCG was measured via pyorgallol autoxidation spectrophotometry. The concentration of pyrogallol acid was 60nmol • L-1 , and detect condition were set to pH=7.3, wavelength was 319nm. The concentration of reaction liquid system (EGCG: =1:1) were set 5, 10, 50, 100, 250, 500μmol • L-1 to investigate the effects of EGCG concentration while the ratios between EGCG and Cu2+ were set as 1:4, 1:3, 1:2, 2:1, 3:1, 4:1 to investigate the effects of different ratios.

III. Results and Discussion

A. Redox character in the mixed system (EGCG: Cu^{2+}=1:1) at different concentrations.

Redox potential of EGCG can be seen in Fig .1. When concentration of EGCG are 5μmol•L-1and 10μmol•L-1 it present prooxidant, while concentrations are 50μmol•L-1, 100μmol•L-1, 250μmol•L-1, 500μmol•L-1 showed antioxidant, respectively. Different concentrations of EGCG have a greater difference in redox property, it showed significant concentration-dependent, low concentrations present prooxidation while high concentrations of EGCG shows antioxidant. Although EGCG of 5μmol•L-1 and 10μmol•L-1 treatment group exhibited a pro-oxidant effect, there was no significant difference (P = 0.95> 0.05) between the two group in pro-oxidant capacity. Arranged antioxidation by concentrations, respectively, 50μmol•L-1 1> 500μmol•L-1> 250μmol•L-1≈100μmol•L-1. Song [40] on his study shows auto-oxidation of EGCG will produce higher concentrations of hydrogen peroxide in the medium, so we speculate that in 5μmol•L-1 and 10μmol•L-1 concentration, ECGG showed pro-oxidant effect may be related to hydrogen peroxide which produced in the process, because, as a strong oxidant, hydrogen peroxide generating will enhance oxidation of liquid.

Fig .1 Shows oxidation characteristics under different concentration of the mixed system. In six kinds of concentrations (5μmol•L-1, 10μmol•L-1, 50μmol•L-1, 100μmol•L-1, 250μmol•L-1, 500μmol•L-1), the effect of Cu2+ was not significant (P> 0.05) in the concentration of 5,10μmol•L-1 and 50μmol•L-1, but in 100μmol • L-1, Cu2+ enhances the antioxidant character of EGCG significantly (P <0.05). However, when concentration is 250μmol•L-1, effect of Cu2 + is not significantly in the front 50 minutes, but improved antioxidant of EGCG after. Similarly, Cu2+ reduced antioxidant of EGCG before 90minutes, improving the antioxidant activity of EGCG after when concentration is 500μmol•L-1.

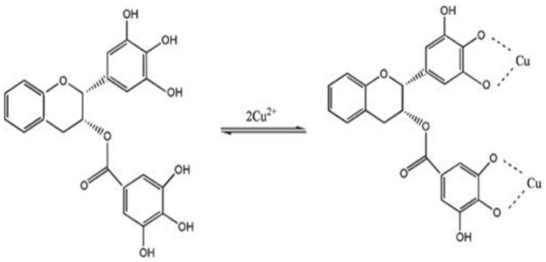

Figure 1. The hypothetical scheme of reaction between Cu2+and EGCG.

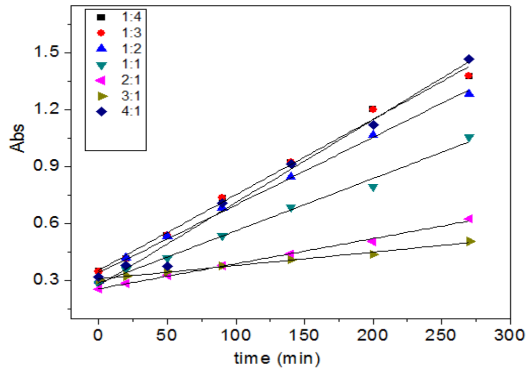

Figure 2. Absorbance results fit a linear change over time in different ratios between EGCG and Cu2+

As can be seen from Table 1, curve fitting results R2 value is high, and the slope can be approximated as an evaluation for the rate of oxidation. Anti-oxidation was enhanced with increasing concentration when the concentration is above 100μmol•L-1. It presented that there a positive correlation between the accession of copper ions concentration and antioxidation in reactant solution. Copper ion played an important role for antioxidant of EGCG. Fig .2 shows the process of copper ion react with EGCG, two copper ions can bind two phenolic hydroxyl at EGCG molecule’s A ring and C ring to form a stable complexes, making the A ring and C ring left only one free hydroxyl group, which makes the EGCG antioxidant character becomes stable.

TABLE I. Curves fitting results in different concentrations of Cu2 +: EGCG (1:1) mixed solution.

Concentration ($\mu mol\cdot L^{-1}$)	5	10	50	100	250	500
Slope	**0.0045**	**0.0042**	**0.0024**	**0.0022**	**0.0016**	**0.0011**
R^2	**0.9592**	**0.9898**	**0.9901**	**0.9970**	**0.9948**	**0.8917**

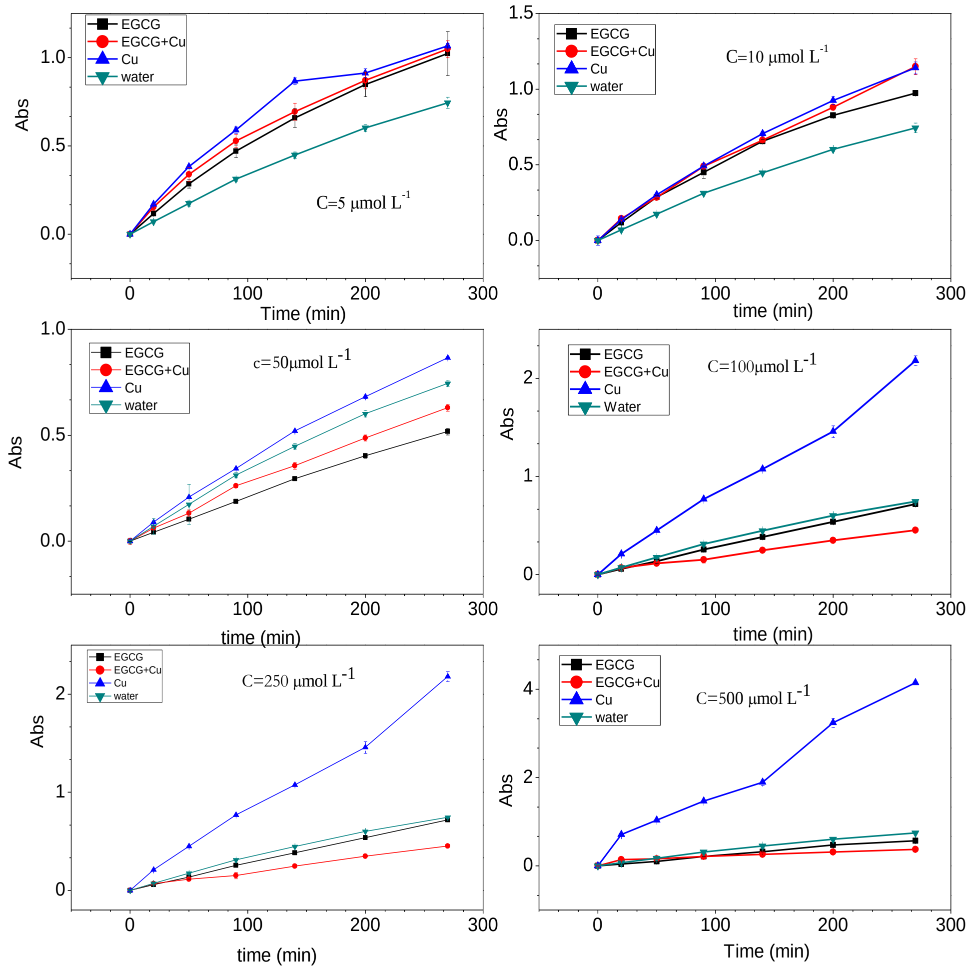

Figure 3. Absorbance changes over time in different concentrations of Cu2+: EGCG (1:1):

TABLE II. ABSORBANCE CHANGES OVER TIME IN A LINEAR FITTING IN DIFFERENT RATIOS OF EGCG AND CU2+

EGCG:Cu^{2+}	1：4	1：3	1：2	1：1	2：1	3：1	4：1
Slope	0.0040	0.0040	0.0036	0.0028	0.0013	0.0007	0.0044
R^2	0.992	0.992	0.995	0.992	0.995	0.990	0.980

B. Redox character in the mixed system (EGCG: Cu^{2+}=1:1) at different concentrations.

Average oxidation of different mixed systems of different ratios between EGEG and Cu2+ can be obtained from Fig .4. Linear fitting results (table 1) showed curves fitting R2 of 0.9867 < 0.99 in ratio of 4:1 while other various ratios of fitting R2 were greater than 0.99, indicated that we can use the curve's slope to measure different ratios of the average size of oxidation, slopes of treatment group is: k1:4 > k4:1 > k1:3 > k1:2 > k1:1 > k2:1 > k3:1.

Fig .3 illustrates antioxidant of EGCG in Different proportion of EGCG: Cu2+ solution. We can see the slope of the curves in treatment groups were less than control group of Cu2+, present that it decrease oxidation of Cu2+ after adding EGCG. However, the slope of curves are greater than the water control group when ratios between EGCG and Cu2+ are 1: 4, 1: 3, 1: 2 and 4: 1, respectively, it indicated that the four mixed solutions still showed prooxidation. While slope of curves is less than the control group at ratios is 1: 1, 2: 1 and 3: 1, and presented antioxidation. The curve of 1:1 treatment group of EGCG and Cu2+ close to the curve of control group, presented weak antioxidant capacity. In the treatment group， the slope of curve was much lower than that of pure water control, showed strong antioxidant activity. It showed that antioxidation capacity enhanced with the increases in concentration of EGCG and the mixed solution in a ratio of 3: 1 presented the strongest antioxidant.

IV. CONCLUSIONS

Different concentrations of EGCG have a greater difference characters, it showed significant concentration-dependent, low concentrations present prooxidation while high concentrations of EGCG shows an antioxidant activity. It need more deeply research to explore the reason why EGCG have concentration-dependent in redox. Meanwhile copper ion played an important role for antioxidant of EGCG, because cooper ion can react with EGCG form a stable complex which makes the EGCG antioxidant character becomes stable. And the average rate of oxidation in different ratios between EGCG and Cu2+ measured by slope of curve is: k1:4 > k4:1 > k1:3 > k1:2 > k1:1 > k 2:1 > k3:1 more further researches are needed to investigate the effects on antioxidation of mixed solution in different ratios between EGCG and copper ion.

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