CNIC-01374
SMC-0151

中国核科技报告

CHINA NUCLEAR SCIENCE AND TECHNOLOGY REPORT

某些中药活性成分抗氧化和清除自由基作用的研究

ANTIOXIDATION AND SCAVENGING EFFECTS OF SOME EXTRACTS FROM CHINESE MEDICINES ON FREE RADICALS

中国核情报中心
原子能出版社

China Nuclear Information Centre
Atomic Energy Press

王崇道：苏州医学院副教授，1967年毕业于苏州医学院医疗系。

Wang Chongdao: Associate professor of Suzhou Medical College. Graduated form Medical Department, Suzhou Medical College in 1967.

CNIC-01374
SMC-0151

某些中药活性成分抗氧化和清除自由基作用的研究

王崇道　强亦忠　劳勤华　崔风梅
（苏州医学院放射医学研究所，苏州，215007）
邵　源　孙存普
（北京放射医学研究所生物医学 ESR 实验室，北京，100850）

摘　要

采用改良的 TBA 分光光度法研究知母宁、槲皮素与单宁酸对辐射所致小鼠组织中 LPO 含量升高的抑制作用。用 ESR 方法研究了上述三种制剂对辐照所致自由基的清除作用。结果显示，知母宁、槲皮素的抗氧化作用优于单宁酸，而清除自由基作用三者相仿。

Antioxidation and Scavenging Effects of Some Extracts from Chinese Medicines on Free Radicals

WANG Chongdao QIANG Yizhong LAO Qinhua CUI Fengmei
(Institute of Radiation Medicine, Suzhou Medical College, Suzhou 215007)
SHAO Yuan SUN Cunpu
(Biomedical ESR Laboratory, Beijing Institute of Radiation Medicine,
27 Taiping Rd, Beijing 100850)

ABSTRACT

The inhibiting effects of Chinonin, Quercetin and Tannic Acid on the lipid oxidation induced by radiation exposure were investigated by means of a modified TBA spectrophotometry. The scavenging effects on free radicals caused by γ-irradiation exposure of the three active principles were observed by technique of ESR. The results showed that antioxidation effects of Chinonin and Quercetin were better than that of Tannic Acid, while the scavenging effects of the three active principles on free radicals were similar.

INTRODUCTION

A great quantity of free radicals can be produced when organism is exposed to ionization radiation. It is known that radiation damage is closely relevant to free radicals [1]. Free radicals including active oxygen free radicals play an important role in radiation damage, senility, malignant tumors and immunity diseases [2]. An attack of free radicals upon biomacromolecules is another important factor in the mechanism of radiation injuries in addition to radiation direct effects on cells[3]. Moreover, lipid peroxides (LPO) induced by free radicals are related to the damage of cell membranes and organelles [4]. Recently it was reported that active oxygen free radicals can cause mutation and apoptosis[5~7]. In order to prevent the damage of free radicals produced by ionization radiation, it is critical to find safe and efficient scavengers for protection against radiation damage.

In this paper the scavenging effects of the three active principles from traditional Chinese medicines i.e. Chinonin, Quercetin and Tannic Acid and the comparison of their effects were studied so as to lay theoretical foundation for use of the principles in clinic therapy and prevention of radiation sickness.

1 MATERIALS AND METHODS

1.1 Materials

Chinonin was purchased from Southern Pharmaceutical Factory (Guangzhou, China) (Grade 98%), Tannic Acid was purchased from Zunyi No.2 Chemical Plant (Guizhou, China) (Grade 99%), Quercetin was purchased from Beijing Chemical Plant (Beijing, China) (Grade 99%). 1, 1, 3, 3-Tetra Ethoxgenpropane (TEP) and Thymidine-5'-monophosphate (5'-TMP) (free acid, Grade 99%, MW322.2) were purchased from Sigma Chemical Co., T-Nitrosobutane (t-NB) (MW 174.24) was purchased from Aldrich Co. KM mice (male, weight 20 ± 2 g) were offered by Experimental Animal Raising Center., Suzhou Medical College.

1.2 Methods

1.2.1 Effects of the three active principles on lipid oxidation induced by radiation exposure

After exposed to whole-body γ-irradiation (total dosage 3 Gy, dose rate 1 Gy/min), the mice were given the three active principles, separately. They were administered twice per day for consecutive 3 days by intragastric perfusion. The dosage of Chinonin was 0.3 ml of 0.06% Chinonin aqueous solution each time (equal to 3.5 mg of Chinonin/day • mouse), Tannic Acid was 0.3 ml of 2.5% Tannic

Acid aqueous solution each time (equal to 15 mg of Tannic Acid/day • mouse), and Quercetin 0.3 ml of 0.2% Quercetin aqueous solution each time (equal to 1.2 mg of Quercetin/day • mouse. The doses of the three active principles administered to the mice were calculated from the respective human's conventional effective doses. The controls were given 0.3 ml of distilled water.

The LPO contents in the mouse tissues (liver, spleen and kidney) were determined by a modified TBA spectrophotometry [8], in which 0.8% TBA acetic acid solution and butanol solution were substituted for TBA aqueous solution and butanol-pyridine solution in the former method. The LPO contents in the mouse tissues were measured by a Model-722 spectrophotometer (Shanghai, China), and expressed in nmol (MDA)/g (wet weight).

1. 2. 2 ESR research of the three active principles into scavenging free radical induced by radiation exposure

T-NB was dissolved into 20 nmol/L aqueous solution by adding of redistilled water, then turned into light blue solution by stirring for 24 hours under the condition of no light exposure. After that 5'-TMP and t-NB were mixed with Chinonin, Quercetin and Tannic Acid of different concentrations until the final concentrations of 5'-TMP and t-NB were 5 mmol/L and 10 mmol/L, respectively. Chinonin concentration was 0.02, 0.1 and 0.5 mg/ml; Quercetin: 0.1, 0.5 and 2.5 mg/ml; and Tannic Acid: 0.5, 2.5 and 12.5 mg/ml, respectively. After N_2 was ventilated for an hour, the tubes were sealed. All the tubes were exposed to ^{60}Co-γ-ray at room temperature with total dosage of 100 Gy, dose rate 212～216 Gy/min. As soon as the exposure was completed, ESR spectra were measured (Bruker ESP300-ESR spectrometer, Bruker CO. Germany) so as to identify characteristic spectra and signal amplitudes of ESR. The parameters for ESR measurement were as follows: modulation frequency: 25 kHz, modulation range: 0.1 mT, scanning width: 10 mT, microwave power: 10 mW, time constant: 10.24 s, scanning time: 41.94 s, central magnetic field: 347 mT, magnetic field frequency: 9.70 GHz.

1. 2. 3 Statistical analysis

The LPO contents were expressed in mean±standard deviation. The difference of LPO content among each group was examined by means of t-test. $P<0.01$ was taken as a criterion for statistically very significant difference, $P<0.05$—statistically significant difference, $P>0.05$ — statistically insignificant difference. The signal amplitudes of ESR were represented by the highest peak values in the second group with characteristic spectra signals. The results were expressed in peak value±

standard deviation. Efficiency of each principle on scavenging free radicals was calculated by the following formula:

$$\text{Scavenging efficiency (\%)} = \frac{\text{Mean peak value of the controls} - \text{Mean peak value of the tested}}{\text{Mean peak value of the controls}} \times 100\%$$

The data were analyzed Statistically by t-test with the application of the software program (SAS6: 03).

2 RESULTS

2. 1 Effects of the three active principles on lipid oxidation induced by radiation exposure

The effects of Chinonin, Quercetin and Tannic Acid on changes of LPO contents induced by radiation exposure see Table 1.

Table 1 Effects on lipid oxidation induced by radiation exposure of Chinonin, Quercetin and Tannic Acid

	No. of samples	LPO [nmol (MDA)/g (wet wt)] ($X \pm SD$)		
		Liver	Spleen	Kidney
Control (A)	4	559.18±54.20	448.78±58.93	469.39±44.50
Chinonin (B)	5	252.53±23.45	263.29±43.74	279.28±21.32
Quercetin (C)	7	299.01±21.28	279.61±32.68	295.08±39.67
Tannic Acid (D)	5	405.34±24.19	377.21±14.35	372.30±23.36

Note: A : B, A : C, B : D and C : D, $P<0.01$; A : D, $P<0.05$; B : C, $P>0.05$.

From the Table 1 it was demonstrated that both of Chinonin and Quercetin could dramatically reduce the LPO contents in the liver, spleen and kidney of the tested animals. The differences between the treated and controls were very significant ($P<0.01$). The effect difference between the Tannic Acid group and the controls were significant ($P<0.05$). The efficiency of Chinonin and Quercetin was better than that of Tannic Acid ($P<0.01$), Chinonin and Quercetin had almost the same results ($P>0.05$).

2. 2 ESR research of the three active principles into scavenging free radicals induced by radiation exposure

Chinonin, Quercetin and Tannic Acid demonstrates strong scavenging effects on free radicals caused by radiation exposure (see Table 2, Table 3 and Table 4).

Table 2 The scavenging effect of Chinonin on 5'-TMP free radicals induced by radiation exposure

Chinonin	Signal amplitude of ESR	Scavenging efficiency
mg · ml^{-1}	$X \pm SD$	%
0	2.440±0.180	
0.02	2.250±0.130*	7.79
0.1	1.580±0.320*	37.30
0.5	1.030±0.230*	57.79

*By comparison with the controls: $P<0.05$. No. of the samples in each group: 3

Table 3 The scavenging effect of Quercetin on 5'-TMP free radicals induced by radiation exposure

Quercetin	Signal amplitude of ESR	Scavenging efficiency
mg · ml^{-1}	$X \pm SD$	%
0	2.420±0.380	
0.1	1.110±0.120*	54.13
0.5	1.050±0.220*	56.61
2.5	0.590±0.020*	75.62

*By comparison with the controls: $P<0.01$. No. of the samples in each group: 3.

Table 4 The scavenging effect of Tannic Acid on 5'-TMP free radicals induced by radiation exposure

Tannic Acid	Signal amplitude of ESR	Scavenging efficiency
mg · ml^{-1}	$X \pm SD$	%
0	2.600±0.020	
0.5	1.250±0.020*	51.92
2.5	0.720±0.050*	72.31
12.5	0.580±0.104*	77.69

*By comparison with the controls: $P<0.01$. No. of the samples in each group: 3.

For Chinonin the difference between the controls and the groups with various concentrations of Chinonin were significant ($P<0.05$); for Quercetin and Tannic

Acid the differences between the controls and the groups with various concentrations of Quercetin and Tannic Acid were very significant ($P<0.01$).

The spin trapping signals in ESR spectra of 5'-TMP aqueous system refer to Fig. 1, Fig. 2 and Fig. 3, respectively.

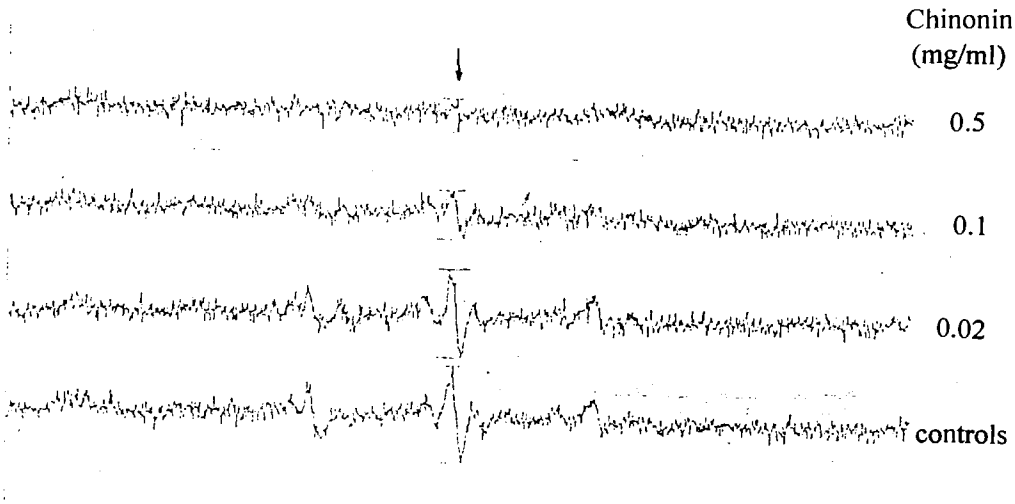


Fig. 1 Spin trapping signals in ESR spectra of the Chinonin-5' -TMP aqueous solution system

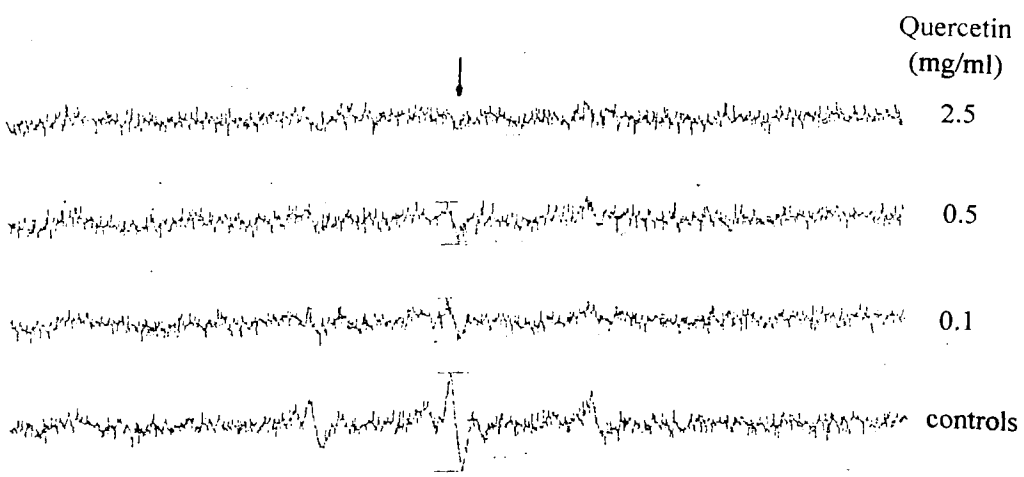


Fig. 2 Spin trapping signals in ESR spectra of the Quercetin-5'-TMP aqueous solution system

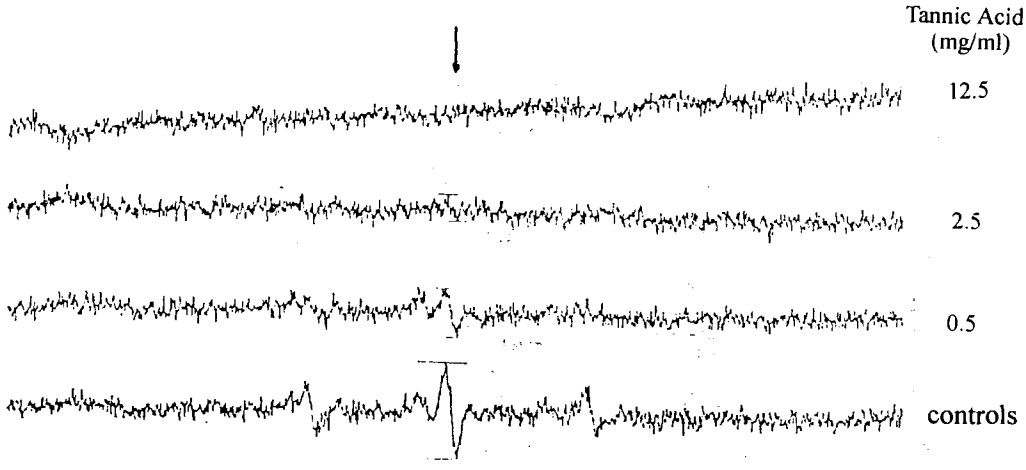
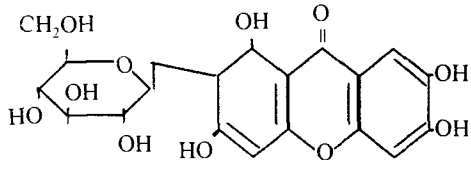


Fig. 3 Spin trapping signals in ESR spectra of the
Tannic Acid-5'-TMP aqueous solution system

3 DISCUSSION

Chinonin is one of the main components extracted from Anemarrhea Asphodeloides Bunge, a sort of traditional Chinese medicine. It is known that Chinonin is a kind of xanthonoid compound with four phenolic hydroxyls, and its structural formula of chemistry is as follows [9]:



From the structure formula it is suggested that Chinonin has a strong antioxidation effect[10]. Our experiments confirmed that Chinonin had a good antioxidation effect when it was given 0.3 mg/d • mouse for consecutive 3 days, the LPO content in the mouse liver, spleen and kidney reduced by 54.84%, 46.13% and 40.50%, respectively in comparison with those that in the controls ($P<0.01$). Chinonin had a strong scavenging effect on free radicals induced by radiation exposure, too. In its lower concentration (0.5 mg/ml), scavenging efficiency of Chinonin on free radicals reached over 50% (Table. 2, Fig. 1).

Quercetin is one of natural flavonoids. It was reported in some literature that Quercetin could protect the damage from oxygen free radicals (OFR)[11]. It was

demonstrated in our experiments that Quercetin could remarkably reduce the increase of the LPO content in the mouse tissues induced by radiation exposure when it was given 1.2 mg/ day • mouse for consecutive 3 days. The LPO contents in the mouse liver, spleen and kidney dropped by 46.53%, 42.79% and 31.74%, respectively compared with those in the controls ($P<0.01$). The reduce range was similar to that of Chinonin. The ESR research into scavenging free radicals of Quercetin indicated that the scavenging efficiency of Quercetin was almost the same as that of Chinonin in concentration of 0.5 mg/ml, but Quercetin's efficiency increased to 70% when its concentration was increased to 2.5 mg/ml, which showed that Quercetin had a good scavenging effect on free radicals, too (see Table 3, Fig. 2).

Tannic Acid, also called Tannin, is the main component of tea. Tannic Acid can produce catacholamine, which possesses many phenolic hydroxyl groups. Based on our experiment results as far antioxidation was concerned the effect of Tannic Acid was not as good as that of Chinonin and Quercetin. Even so, when 15 mg of Tannic Acid per day per mouse was administered, the LPO contents in the mouse liver, spleen and kidney diminished by 27.54%, 22.83% and 20.68%, respectively compared with those in the controls ($P<0.05$). On basis of ESR measurement of the scavenging effect on free radicals the scavenging efficiency of Tannic Acid is similar to that of Chinonin and Quercetin when its concentration is 0.5 mg/ml. Because Chinonin slightly dissolved in water, its concentration could not easily be increased so that its scope of application is restricted unless some surface activators are added to increase its dissolution, while Tannic Acid was easily dissolved in water, so the scavenging efficiency could get to more than 70% when its concentration reached 12.5 mg/ml (see Table 4, Fig. 3).

Deoxyribonucleic Acid (DNA) is one of biomacromolecules, which is most sensitive to radiation exposure [12]. Free radicals are relevant to the damage of DNA. The agents of radiation protection can capture, inactivate free radicals, and alleviate the damage of DNA as a consequence of the transmittance of unpaired electrons of DNA free radicals, scavenge and repair of the free radicals, thus the structure and functions of DNA are protected [13]. Based on our research, it is inferred that the antioxidation and scavenging effects of Chinonin, Quercetin and Tannic Acid on free radicals induced by radiation exposure were closely related to capture and inactivation of free radicals.

In summary, the three active principles have strong antioxidation and

scavenging effects. The antioxidation effects of Chinonin and Quercetin are better than that of Tannic Acid. Chinonin, Quercetin and Tannic Acid have almost the same scavenging effects on free radicals induced by radiation exposure. The scavenging efficiency of Tannic Acid can further increase with the rise of its concentration for its higher dissolution in water.

REFERENCE

1 Simic M G, Bergtolo D S, Karam L R. Generation of oxygen radicals in biosystems. Mutat Res., 1989, 214: 3～12

2 Bulkley G B. The role of oxygen free radicals in human disease processes. Surgery , 1983, 94: 407～411

3 Halliwell B, Gutteridge J M C. Free radicals in biology and medicine. Oxford: Clarendon press, 1989. 196～276

4 Konings A W T, Trieling W B. The inhibition of DNA sythesis in vitamin-E-depleted lymphosarcoma cells by X-rays and cytostatics. Int . J. Radiat., 1977, 31: 397～400

5 Reid T M, Loeb L A. Mutagenic specificity of oxygen radicals produced by human leukemia cells. Cancer Res., 1992, 52: 1082～1086

6 Wood M L, Dizdaroglu M, Gajewski, et al. Mechanistic studies of ionizing radiation and oxidative mutagenesis: Genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry. 1990, 29: 7024～7032

7 Halliwell B, Gutteridge J M C. The role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol., 1990, 186:1～85

8 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 1979, 95: 351～358

9 Qiu Q F, Mei N Y, Zhang J H, et al. The study of electron spin transfer from γ-irradiated DNA to Chinonin. J. Radiat. Res. Radiat. Proces., 1995, 13: 249～251

10 Zhang H Y, Wang F Y, Li Ming, et al. Studies of scavenging effects on active oxygens of Chinonin. J. Radiat. Res. Radiat. Proces., 1997, 15: 224～227

11 Husain S R, Cillard J, Cillard O. Hydroxyl radical scavenging activity of Havonoids. Phytochemistry, 1987, 26: 2489～2495

12 Halliwel L B, Aruoma O I. DNA damage by oxygen-derived species, its mechanism and measurement in mammalian systems. FEBS lett, 1989, 281: 9～19

13 Zastawny T H, Czerwinska B, Drzewieska, et al. Radiation induced oxidative DNA base damage and its repair in liver chromatin DNA of rats upon whole body gamma-irradiation. Acta Biochim pol, 1996, 43: 579～582

图书在版编目（CIP）数据

中国核科技报告 CNIC-01374, SMC-0151：某些中药活性成分抗氧化和清除自由基作用的研究：英文/王崇道等著. —北京：原子能出版社，1999.9

ISBN 7-5022-2077-1

I. 中… II. 王… III. 核技术-研究报告-中国 IV. TL-2

中国版本图书馆 CIP 数据核字 (1999) 第48605号

原子能出版社出版 发行

责任编辑：武 洁

社址：北京市海淀区阜成路 43 号 邮政编码：100037

中国核科技报告编辑部排版

核科学技术情报研究所印刷

开本 787×1092 mm 1/16 印张1/2 字数10千字

1999年11月北京第一版 1999年11月北京第一次印刷

印数：1—200

定价：5.00 元

CHINA NUCLEAR SCIENCE & TECHNOLOGY REPORT

ISBN 7-5022-2077-1