

Full Length Research Paper

Powerful protective effects of gallic acid and tea polyphenols on human hepatocytes injury induced by hydrogen peroxide or carbon tetrachloride *in vitro*

Tiejing Li, Xin Zhang and Xinhui Zhao*

Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, 150030 Harbin, People's Republic of China.

Accepted 9 December, 2009

Gallic acid and tea polyphenols are found in tea and considered as natural antioxidants. In this study, the cytoprotective effects of gallic acid and tea polyphenols against human hepatocytes (HL - 7702 cell line) oxidative injury induced by hydrogen peroxide (H₂O₂) or carbon tetrachloride (CCl₄) were evaluated *in vitro*, with α -tocopherol as standard antioxidant. Some biochemical assays were carried out to determine the cytoprotective effects of gallic acid and tea polyphenols on the hepatocytes subjected to oxidative injury, including cell viability, the content of reduced glutathione in cells, lactate dehydrogenase leakage into culture medium and the formation of malondialdehyde in the cells. Statistical results showed that pretreatment of the cultured cells with gallic acid, tea polyphenols or α -tocopherol at concentrations of 5, 10, 20 mg/l for 30 min before oxidative injury of the cells could provide cytoprotective effect to the cells to improve cell viability, increase the content of reduced glutathione in cells, reduce lactate dehydrogenase leakage into culture medium and decrease the formation of malondialdehyde in the cells. It was shown that gallic acid and tea polyphenols displayed stronger cytoprotective effect against oxidative injury of the hepatocytes than α -tocopherol at same addition level and gallic acid was the most powerful compound.

Key words: Gallic acid, tea polyphenols, hepatocytes, oxidative injury, hydrogen peroxide, carbon tetrachloride.

INTRODUCTION

Polyphenols are widely found in the plant kingdom and especially rich in some foods and medicinal plants, and considered as natural antioxidants. For example, tea polyphenols are important components of black or green tea and have strong antioxidation. Gallic acid exists as part of tea polyphenols and epicatechin or epigallocatechin gallate are the main components in tea polyphenols. In recent years, natural antioxidants, especially those in natural foods or medical plants, draw attention of many food or medicine researchers for these compounds may be daily consumed and have many health functions. It was found in many studies that polyphenols exhibit some helpful biological effects such

as antioxidative, anticarcinogenic, anti-hepatotoxic, anti-ischaemic, anti-allergic, anti-ulcerative and anti-inflammatory activities (Shahidi and Naczki, 2004). Polyphenols such as flavonoids might exert effects such as antioxidants, free radical scavengers and chelators, and may be involved in preventing free radical induced cytotoxicity and lipid peroxidation that are associated with cell aging and chronic diseases (Havsteen, 2002). Previous studies have revealed that polyphenols exhibit clear cytoprotective effect on rat or tumour hepatocytes injury system (Sugihara et al., 1999; Lima et al., 2006; Yao et al., 2007), and our study results also showed that three flavonoids could protect human hepatocytes against oxidative injury induced by hydrogen peroxide (H₂O₂) or carbon tetrachloride (CCl₄) *in vitro* (Zhao and Zhang, 2009), but the protective effects of gallic acid and tea polyphenols against oxidative injury of human hepatocytes is also needed to be studied.

*Corresponding author. E-mail: zhaoxh@mail.neau.edu.cn. Tel: +86 451 5519 1813. Fax: +86 451 5519 0340.

Liver is the major organ in our body involved in the biotransformation of exogenous chemicals and is the target for toxic substances. During the detoxification of xenobiotics, reactive oxygen species (ROS) are generated which induce production of lipid peroxides within the hepatocytes and subsequently cause oxidative stress, cell death and ultimately result in liver disease, such as hepatocellular carcinoma, viral and alcoholic hepatitis and non-alcoholic cirrhosis (Kohen and Nyska, 2002; Vitaglione et al., 2004). Therefore, scavenging of ROS or protection against oxidative stress in the liver is very important process. Cytoprotective effect of polyphenols against oxidative injury of human hepatocytes is beneficial to keep the liver healthy.

It was reported that hepatocytes or hepatocyte cell lines in culture keep many metabolic enzyme characteristics of the intact liver *in vivo*, and are served as ideal models *in vitro* to study or screen substances with hepatoprotective activity (Hengstler et al., 2000; Runge et al., 2000). In the presented study, the cytoprotective effect of gallic acid and tea polyphenols against the oxidative injury induced by H₂O₂ or CCl₄ was studied on human hepatocytes (HL-7702 cell line), isolated from normal human liver tissue, with α -tocopherol as a standard antioxidant. To examine and compare the cytoprotective effect of gallic acid and tea polyphenols on hepatocytes, cell viability, lactate dehydrogenase (LDH) leakage into culture medium, malondialdehyde (MDA) formation and the content of reduced glutathione (GSH) in hepatocytes were determined, for these biochemical indices are considered as important indicators of cell injury and lipid peroxidation and widely applied in past studies. The aim of this study is to provide some experimental supports to the health benefits of tea polyphenols or tea and reveal the importance of natural and biological antioxidants in foods.

MATERIALS AND METHODS

Chemicals

Gallic acid and tea polyphenols were purchased from Shanghai Kangjiu Chemicals Co., Ltd. (Shanghai, China, purity \geq 98%). The following reagents were from Solarbio Science and Technology Co., Ltd (Beijing, China): α -tocopherol (purity \geq 95%), fetal bovine serum (FBS), RPMI-1640 medium, 3-[4,5-dimethylthiazol-2-yl]-2,5-dephenyl tetrazolium bromide (MTT), reduced glutathione (GSH), reduced nicotinamide adenine dinucleotide (NADH), 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), dimethylsulfoxide (DMSO), 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), Triton X -100 and sodium pyruvate. All other chemicals were analytical agents.

Cell culture

All experiments and following evaluations were conducted in the period of 2007 - 2009 at Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin. Hepatocytes (HL - 7702 cell line) were obtained from Shanghai Institutes for Biological Sciences (Shanghai, China) and cultured in RPMI-1640 medium containing 10% FBS, 100 U/ml penicillin G and 100 mg/ml

streptomycin at 37°C in an incubator of humidified air with 5% CO₂. The sub-confluent cells (80%) were passaged with a solution containing 0.25% trypsin and 0.02% EDTA. The cells were seeded onto 96-well plates at 1×10^4 cells/well for cell cytotoxicity and viability assay, or 6-well plates at 1×10^6 cells/well for MDA, LDH and GSH determination.

Cell treatment

Gallic acid, tea polyphenols and α -tocopherol were dissolved in DMSO and mixed well with the medium so that the final concentration of DMSO was not \geq 0.1% (v/v). The cells in test groups were incubated with each compound first at 5, 10, 20 mg/l for 30 min and then oxidative injured with H₂O₂ (6.5 mmol/L, 1.5 h) or CCl₄ (8 mmol/L, 6 h). The cells in control group, containing equal volume of DMSO but without any addition of the tested compounds, were not oxidative injured. The cells in model group, containing equal volume of DMSO but without any addition of the tested compounds, were oxidative injured. Then, all groups were processed immediately for biochemical assays.

Biochemical assays

Cell viability

Cell viability of the hepatocytes was measured by MTT assay. After the cells were treated as above, twenty microliter of MTT solution (5 g/L) was added to each well of the plate. After 4 h of incubation, the plate was centrifuged at 1800 g for 5 min at 4°C and the MTT solution was removed from the wells by aspiration. One hundred microliter of DMSO was added to each well to resolve the formazan generated from MTT (Wu et al., 2007). The absorbance of each well was recorded on a microplate reader (Bio Rad, Hercules, CA, USA) at the wavelength of 490 nm. Cell viability in each test group and model group was expressed as percentage of the control group. Cells in control group were as 100% viable.

LDH leakage

After cell treatment mentioned above, the culture medium was centrifuged at 50 g for 1 min and the supernatant was collected. One hundred microliter of the supernatant was mixed with 1 ml of sodium pyruvate (1.22 mmol/L) in 50 mmol/L phosphate buffer (pH 7.5) and 20 μ l of NADH (6.2 g/L) (Dvořák et al., 2003). The decrease in absorbance at 340 nm over 3 min was measured and calculated. LDH leakage was expressed as the ratio of the decreasing rate of absorbance per minute in test group or model group to the decreasing rate of absorbance per minute in control group.

MDA assay

The formation of MDA in the cells was measured by the thiobarbituric acid (TBA) method as described by Sahu et al. (2006). Briefly, a 0.5 ml aliquot of the cell suspension was added to 0.5 ml of the reaction medium containing TBA (0.375%, w/v) and trichloroacetic acid (15%, w/v) in 0.25 mol/L HCl. The mixture was heated in boiling water for 15 min, cooled to ambient temperature and centrifuged to remove insoluble materials. The supernatant was measured at 532 nm using 1, 1, 3, 3-tetraethoxypropane as standard and expressed as mmol/L MDA/g proteins. Protein content was determined by Lowry's method using bovine serum albumin as standard protein.

Table 1. Cytotoxicity of gallic acid and tea polyphenols on the hepatocytes incubated for 24 h.

Compounds	Cell viability at three concentrations (%) ¹		
	5 mg/l	10 mg/l	20 mg/l
Gallic acid	99.8 ± 1.4	98.7 ± 1.3	93.9 ± 1.6 ^a
Tea polyphenols	102 ± 2.8	99.6 ± 1.4	99.1 ± 1.6

¹Cell viability in control group was as (100 ± 1.4) %. The number of trial times was three. All values are expressed as mean ± SD. Upper letters a, P < 0.01, comparing with the cell viability in control group.

GSH assay

The hepatocytes were washed and harvested in 0.5 ml of PBS with 0.1% of Triton X - 100. After 10 min of incubation, the mixture was centrifuged (3000 g, 10 min, 4°C) and 0.3 ml of the supernatant was mixed with 1.0 ml of Tris-base (0.8 mol/l)-EDTA (0.02 mol/l) buffer, pH 8.9. Following the addition of 0.1 ml of DTNB (0.01 mol/L) in methanol (Dvořák et al., 2003). The content of reduced GSH in the cells was measured at 412 nm and expressed as mg GSH/g proteins. Protein content was determined by Lowry's method using bovine serum albumin as standard.

Morphology observation

Morphology of the hepatocytes in different groups was observed and photographed with an inverted photomicroscope (Model AE31, Motic, Germany), equipped with a photographic system at 100 × magnification.

Statistical analysis

All data were expressed as mean ± standard deviation (SD) of at least three independent assays. The differences between the mean values of multiple groups were analyzed by one-way analysis of variance (ANOVA) with Duncan's Multiple Range Test. ANOVA data with a P < 0.05 were classified as statistically significant. SPSS 13.0 and Excel 2003 software were used to analyze and report the data.

RESULTS

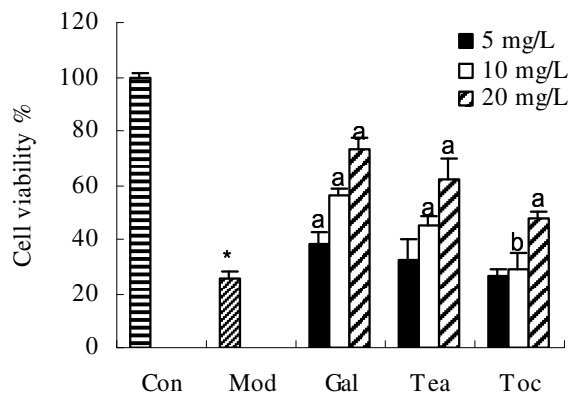
Gallic acid and tea polyphenols showed little toxic effect on the hepatocytes (HL-7702 cell line) when the cells were exposed to them at three concentrations for culture periods of 24 h and assessed via the MTT assay. The assay results (Table 1) indicated that tea polyphenols showed no cytotoxicity to the cells at three concentrations applied in pretreatment. Significant cytotoxicity was observed when the addition concentration of gallic acid was up to 20 mg/l, but cell viability was still maintained at above 90%, which meant that shorter exposure time of the cells to gallic acid would have little adverse effect on cell viability. We pretreated the hepatocytes with gallic acid or tea polyphenols for 30 min in proceeding experiments. Therefore, the cytoprotective effects of gallic acid and tea polyphenols on the hepatocytes were evaluated at three concentrations, 5, 10 and 20 mg/l.

Effects of gallic acid and tea polyphenols on H₂O₂ or CCl₄ induced hepatocytes injury

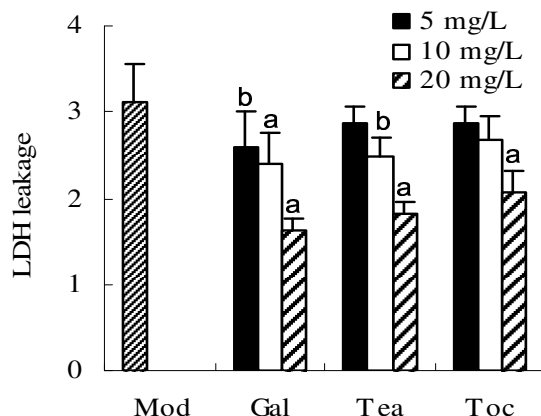
Comparing to the cells in control group, treatment of the hepatocytes with H₂O₂ (6.5 mmol/L for 1.5 h) or CCl₄ (8 mmol/L for 6 h) resulted in a significant decrease in cell viability in model group and all test groups (P < 0.01), as shown in Figure 1A (hepatocytes injured by H₂O₂) and 1B (hepatocytes injured by CCl₄). The cell viability in model group decreased to 26.0% (hepatocytes injured by H₂O₂) or 25.0% (hepatocytes injured by CCl₄), indicating that oxidative injury occurred in the hepatocytes. If the hepatocytes were pretreated with gallic acid or tea polyphenols for 30 min, oxidative injury of the cell could be alleviated, especially when gallic acid or tea polyphenols was added at higher level 20 mg/l (p < 0.01). Cell viability in most test groups was enhanced clearly (p < 0.01 or p < 0.05). When gallic acid addition was 20 mg/l, cell viability enhanced to 73.2 or 74.2% (H₂O₂ or CCl₄ injured). Meanwhile, when tea polyphenols addition was 20 mg/l, cell viability was enhanced to 62.0 or 55.1% (H₂O₂ or CCl₄ injured). Gallic acid showed stronger protective effect. α-Tocopherol as reference antioxidant also showed similar protective effect against oxidative injury of the hepatocytes, but its effect was weaker than that of gallic acid or tea polyphenols.

Effects of gallic acid and tea polyphenols on H₂O₂ or CCl₄ induced increase of LDH leakage

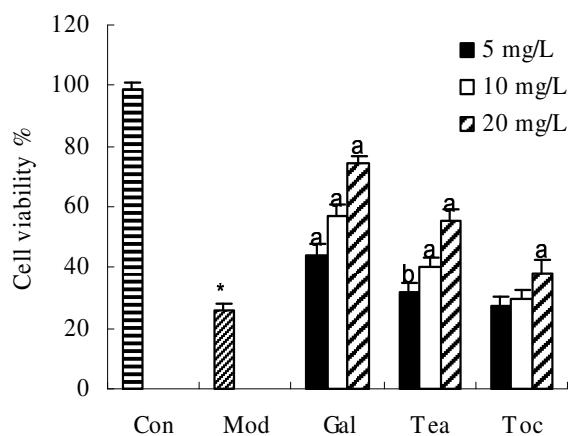
As shown in Figure 2A and 2B, injury of the hepatocytes in model group with H₂O₂ or CCl₄ caused more LDH leakage into culture medium. Pretreatment of the cells with gallic acid or tea polyphenols or α-tocopherol led to the injured cells in test groups with less LDH leakage and this effect was enhanced at higher addition level. When gallic acid, tea polyphenols or α-tocopherol was separately added at higher level (20 mg/l) to the cells, gallic acid could reduce LDH leakage from 3.12 or 2.30 (model group, H₂O₂ or CCl₄ injured) to 1.83 or 1.08 (H₂O₂ or CCl₄ injured), tea polyphenols could reduce LDH leakage to 1.82 or 1.23 (H₂O₂ or CCl₄ injured) and α-tocopherol could reduce LDH leakage to 2.17 or 1.36 (H₂O₂



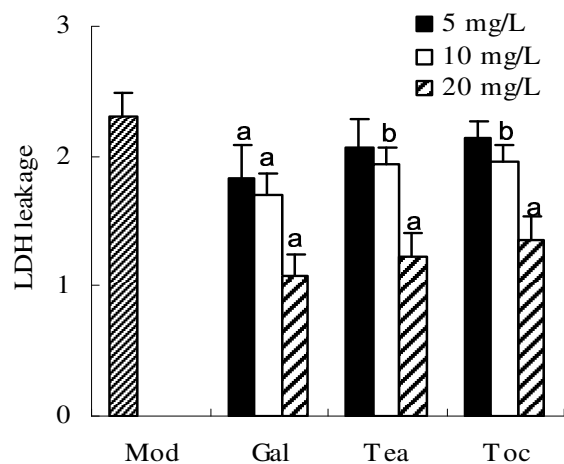
A



A



B



B

Figure 1. The effects of gallic acid, tea polyphenols and α -tocopherol on viability of the hepatocytes injured by addition of H_2O_2 (A) or CCl_4 (B) *in vitro*. The hepatocytes were incubated with gallic acid, tea polyphenols and α -tocopherol for 30 min, then treated with H_2O_2 or CCl_4 for 1.5 h or 6 h. Con, control group; Mod, model group; Gal, gallic acid group; Tea, tea polyphenols group; Toc, α -tocopherol group. The number of trial times was three. Values are expressed as mean \pm SD. Letter a or b, $p < 0.01$ or $p < 0.05$, compared with model group. Star mark, $p < 0.01$, compared with control group.

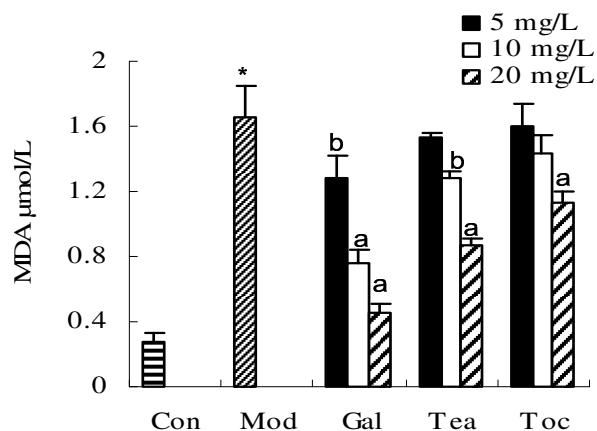
Figure 2. The effects of gallic acid, tea polyphenols and α -tocopherol on LDH leakage of the hepatocytes injured by addition of H_2O_2 (A) or CCl_4 (B) *in vitro*. The hepatocytes were incubated with gallic acid, tea polyphenols and α -tocopherol for 30 min, then treated with H_2O_2 or CCl_4 for 1.5 h or 6 h. Con, control group; Mod, model group; Gal, gallic acid group; Tea, tea polyphenols group; Toc, α -tocopherol group. The number of trial times was three. Values are expressed as mean \pm SD. Letter a or b, $p < 0.01$ or $p < 0.05$, compared with model group.

or CCl_4 injured). Gallic acid showed the strongest protective effect and α -tocopherol showed weaker protective effect.

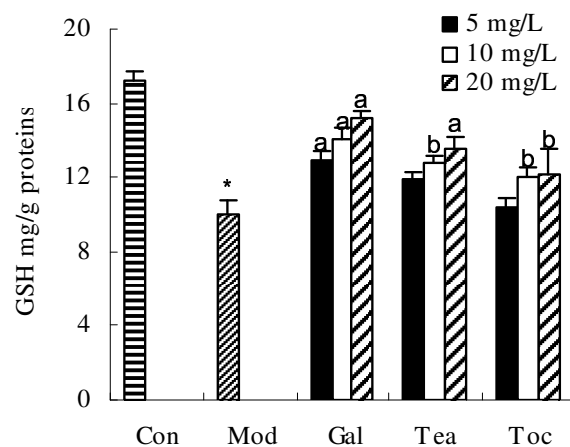
Effects of gallic acid and tea polyphenols on H_2O_2 or CCl_4 induced lipid peroxidation

MDA content in the cells is used as a marker of lipid peroxidation. Comparing to the MDA formation in the hepatocytes in control group, treatment of the hepatocytes with H_2O_2 or CCl_4 led to much more MDA formation in the cells in model group or test groups ($P <$

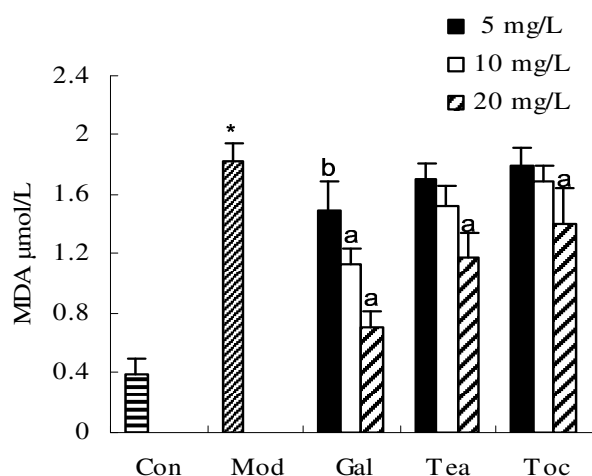
0.01), as shown in Figures 3A and 3B, indicating lipid oxidation occurred in the two groups during cell injury. MDA content in cells in model group was 1.55 or 1.83 mmol/L/g proteins (H_2O_2 or CCl_4 injured); much higher than that in control group (0.27 or 0.40 mmol/L/g proteins). It could be seen that pretreatment of the cells with gallic acid, tea polyphenols or α -tocopherol for 30 min might prevent or delay MDA formation, especially at higher addition levels, for example, at 20 mg/l ($P < 0.01$). When three compound was separately added to the cells at 20 mg/l, the content of MDA decreased to 0.45, 0.87 and 1.13 mmol/L/g proteins (H_2O_2 injured), or to 0.71, 1.18 and 1.40 mmol/L/g proteins (CCl_4 injured), respectively. This result clearly showed that three com-



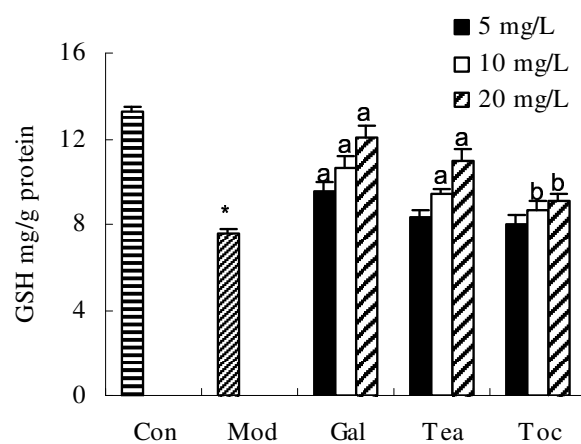
A



A



B



B

Figure 3. The effects of gallic acid, tea polyphenols and α -tocopherol on MDA formation of the hepatocytes injured by addition of H_2O_2 (A) or CCl_4 (B) *in vitro*. The hepatocytes were incubated with gallic acid, tea polyphenols and α -tocopherol for 30 min, then treated with H_2O_2 or CCl_4 for 1.5 h or 6 h. Con, control group; Mod, model group; Gal, gallic acid group; Tea, tea polyphenols group; Toc, α -tocopherol group. The number of trial times was three. Values are expressed as mean \pm SD. Letter a or b, $p < 0.01$ or $p < 0.05$, compared with model group. Star mark, $p < 0.01$, compared with control group.

Figure 4. The effects of gallic acid, tea polyphenols and α -tocopherol on GSH content of the hepatocytes injured by addition of H_2O_2 (A) or CCl_4 (B) *in vitro*. The hepatocytes were incubated with gallic acid, tea polyphenols and α -tocopherol for 30 min, then treated with H_2O_2 or CCl_4 for 1.5 h or 6 h. Con, control group; Mod, model group; Gal, gallic acid group; Tea, tea polyphenols group; Toc, α -tocopherol group. The number of trial times was three. Values are expressed as mean \pm SD. Letter a or b, $p < 0.01$ or $p < 0.05$, compared with model group. Star mark, $p < 0.01$, compared with control group.

pounds displayed their antioxidant effect and had a clear protective effect against H_2O_2 or CCl_4 induced lipid peroxidation in the cells. Gallic acid also showed the strongest protective effect and α -tocopherol showed weaker protective effect.

Effects of gallic acid and tea polyphenols on H_2O_2 or CCl_4 induced depletion of GSH

GSH content in cells also serves as an index to reflect redox status of cells. Comparing to the hepatocytes of

control group, treatment of the hepatocytes with H_2O_2 or CCl_4 significantly decreased GSH content in the cells in model group or in test groups ($P < 0.01$), as shown in Figure 4A and 4B. The decrease of GSH content in the cells resulted from depletion of GSH during oxidative injury of the hepatocytes. Pretreatment of the hepatocytes with gallic acid, tea polyphenols or α -tocopherol for 30 min could significantly provide protective effect against this depletion. Comparing to the cells of model group (9.94 or 7.52 mg/g proteins, H_2O_2 or CCl_4 injured), GSH contents in the cells of test groups were elevated

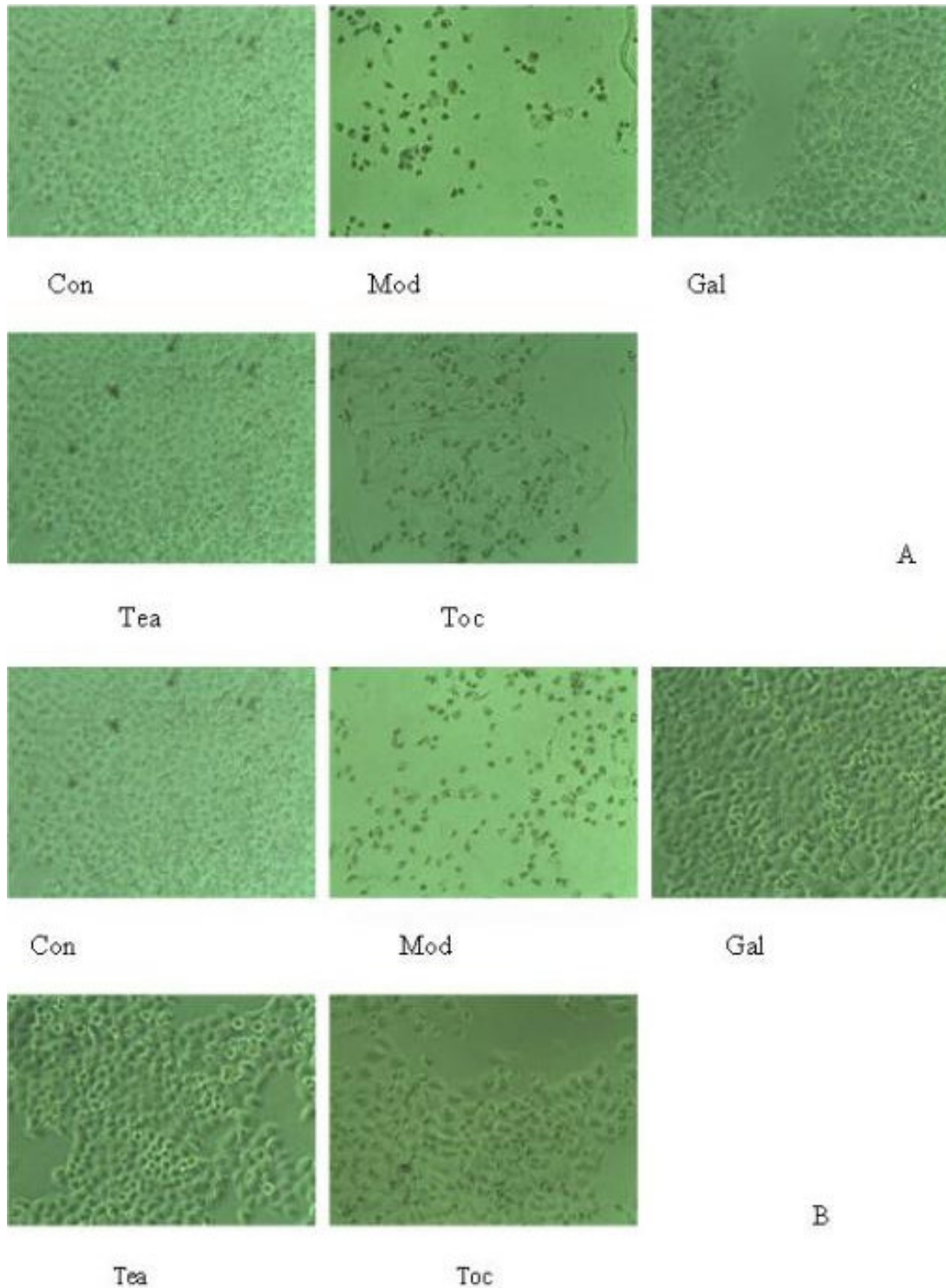


Figure 5. Morphological observations of oxidative injury of the hepatocytes induced by addition of H₂O₂ (A) or CCl₄ (B) *in vitro*. The hepatocytes were incubated with gallic acid, tea polyphenols and α -tocopherol at 20 mg/l for 30 min, then treated with H₂O₂ for 1.5 h or CCl₄ for 6 h. Con, control group; Mod, model group; Gal, gallic acid group; Tea, tea polyphenols group; Toc, α -tocopherol group. Hepatocytes were observed at 100 \times magnification.

were elevated, which were related to the addition levels of gallic acid, tea polyphenols or α -tocopherol. When

three compounds were added separately at 20 mg/l, GSH contents in the cells increased to 14.0, 13.5 and 12.1 mg/g

12.1 mg/g proteins (H_2O_2 injured), or to 12.0, 11.0 and 9.15 mg/g proteins (CCl_4 injured), respectively. The result showed that gallic acid, tea polyphenols and α -tocopherol all had protective effect against H_2O_2 or CCl_4 induced depletion of GSH. Gallic acid also showed the strongest protective effect and α -tocopherol showed weaker protective effect.

Morphological changes of the hepatocytes in culture

Exposure of the hepatocytes to H_2O_2 or CCl_4 injury clear led to significant reduction in cell numbers, as shown in Figure 5A (the hepatocytes injured by H_2O_2) and 5B (the hepatocytes injured by CCl_4). The hepatocytes of model group looked flat, small with fewer aggregates comparing to the cells of control group. The hepatocytes of control groups maintained their typical morphology. Comparing to the cells of model group, pretreatment of the hepatocytes with gallic acid, tea polyphenols and α -tocopherol at three concentrations for 30 min had a significant improvement in cell growth, because the numbers of the cells in test groups increased and more aggregates appeared. Polygonal morphology of the hepatocytes in test groups looked like that in control group, confirming that gallic acid, tea polyphenols and α -tocopherol had clear protective effect against H_2O_2 or CCl_4 induced oxidative injury.

DISCUSSION

H_2O_2 and CCl_4 have highly toxic effect on cells and are often used to induce oxidative stress in cell systems. The mechanism by which CCl_4 causes cell oxidative injury involves that cytochrome P-450 system transforms CCl_4 into CCl_3 and then CCl_3 is transformed into a more reactive CCl_3O_2 , CCl_3O_2 causes lipid peroxidation, disturbs Ca^{2+} homeostasis and eventually kills cells (Farombi, 2000; Wang et al., 2004). H_2O_2 damages the cells by at least two mechanisms. The first is prevented by the anti-oxidants DPPD' and is likely related to the peroxidation of membrane phospholipids. The second is independent of lipid peroxidation and yet dependent upon the continued presence of H_2O_2 (Starke and Farber, 1985). Therefore, elimination of oxidative stress in liver or the hepatocytes with antioxidants might protect liver or the hepatocytes from oxidative injury and insure liver health, which is beneficial to liver to perform its functions.

Our study showed, comparing model group to control group, exposure of the hepatocytes to H_2O_2 or CCl_4 led to cell death, membrane damage, lipid peroxidation and GSH depletion, which were confirmed by some biochemical assays as cell viability, LDH leakage, MDA formation and GSH content in cells. From biochemical assays, it could be seen that gallic acid and tea polyphenols all had protective effect against H_2O_2 or CCl_4 -induced hepatocytes oxidative injury. Gallic acid and tea polyphenols

could improve cell viability, reduce LDH leakage into culture medium, alleviate GSH depletion and inhibit MDA formation in the cells clearly, and showed a dose-dependent protective effect, that is, higher addition levels of the compounds, much protective effect displayed. More importantly, gallic acid and tea polyphenols showed stronger cytoprotective effects against oxidative injury of the hepatocytes than α -tocopherol at same addition level and gallic acid gave most powerful effect. The potential cytoprotective effects of gallic acid, tea polyphenols and α -tocopherol against oxidative injury of the hepatocytes was in the order of gallic acid > tea polyphenols > α -tocopherol.

Cytotoxicity and protective effects of natural antioxidants or plant extracts had been widely studied. In a study about cytotoxicity and protective effect of an aqueous extract of *Rubus chingii* fruits on primary rat hepatocytes, it was shown that 200 mg/l was considered to be feasible concentration of the extract for cell viability, because the viable cells were maintained at above 90% after incubation for 24 h at this concentration (Yau et al., 2002). Based on this conclusion and our experimental result, we also selected gallic acid and tea polyphenols at 5, 10 and 20 mg/l in our study to evaluate their cytoprotective effects against oxidative injury of the hepatocytes, because the cells were exposed to the evaluated compounds only for a short time (30 min) and the viable cells were also more than 90% after incubation for 24 h at all addition levels.

In our previous work, three phenol compounds, kaempferol, quercetin and myricetin, showed their protective effects against oxidative injury of human hepatocytes induced by H_2O_2 or CCl_4 (Zhao and Zhang, 2009), and shared similar protective effects found in the presented work. Quercetin was found to give protective effect against alcoholic oxidative stress on human hepatic tumor cells by reducing LDH leakage into culture medium, alleviating GSH depletion in cells and decreasing the formation of MDA in cells (Yao et al., 2007). Also, cytoprotective effects of some plant extracts against induced oxidative injury of the hepatocytes were reported. The extracts from *Artemisia capillaris* exhibited protective effect on tert-butylhydroperoxide induced oxidative damage in rat primary hepatocytes (Chu et al., 1999), for LDH leakage into culture medium appeared to be reduced, GSH level in the cells to be enhanced and MDA formation in the cells to be decreased. The extract of bilberry displayed cytoprotective effect against primary rat hepatocytes oxidative stress induced by tert-butylhydroperoxide or allyl alcohol (Valentová et al., 2007), which were demonstrated by enhancing cell viability or reducing LDH leakage into culture medium, or decreasing the formation of MDA in the cells. Hepatoprotective effect of the aqueous extract from the leaves of *Acalypha racemosa* was found in CCl_4 -treated rats, for MDA content in the liver decreased when compared with control (Iniaghe et al., 2008). All these results and this presented

work verified the fact that phenol compounds, including gallic acid and tea polyphenols, had protective effects against cell oxidative injury.

Conclusions

As natural occurring antioxidants in tea, gallic acid and tea polyphenols were studied to reveal their protective effects against oxidative injury of human hepatocytes (HL-7702 cell line) induced by H₂O₂ or CCl₄. The hepatocytes were pretreated with gallic acid, tea polyphenols and α -tocopherol for 30 min at 5, 10 and 20 mg/l, then subjected to oxidative injury. Some biochemical assays and morphological observation were carried to determine important indexes of oxidative injury of the cells, including cell viability, the content of GSH in the cells, LDH leakage into culture medium and the content of MDA in the cells. The valuation results showed that gallic acid and tea polyphenols could improve cell viability, increase the content of GSH in the cells, reduce LDH leakage into culture medium and decrease the formation of MDA in the cells significantly, which indicated that they had cytoprotective effect against hepatocytes oxidative injured by H₂O₂ or CCl₄. Gallic acid and tea polyphenols showed stronger cytoprotective effect against oxidative injury of the hepatocytes than α -tocopherol at same addition level and gallic acid gave most powerful effect.

ACKNOWLEDGEMENT

This work was supported by Specialized Research Fund for the Doctoral Program of Higher Education (No. 200802240002) of Ministry of Education, PR China.

REFERENCES

- Chu, CY, Tseng TH, Hwang JM, Chou FP, Wang CJ (1999). Protective effects of capillarisin on *tert*-butylhydroperoxide-induced oxidative damage in rat primary hepatocytes. *Arch. Toxicol.* 73: 263-268.
- Dvořák Z, Kosina P, Walterová D, Šimánek V, Bachleda P, Ulrichová J (2003). Primary cultures of human hepatocytes as a tool in cytotoxicity studies: cell protection against model toxins by flavonolignans obtained from *Silybum marianum*. *Toxicol. Lett* 137: 201-212.
- Farombi EO (2000). Mechanisms for the hepatoprotective action of kolaviron: studies on hepatic enzymes, microsomal lipids and lipid peroxidation in carbon tetrachloride-treated rats. *Pharmacol. Res.* 42: 75-80.
- Havsteen BH (2002). The biochemistry and medical significance of the flavonoids. *Pharmacol. Ther.* 96: 67-202.
- Hengstler JG, Utesch D, Steinberg P, Platt KL, Diener B, Ringel M, Swales N, Fischer T, Biefang K, Gerl M, Bottger T, Oesch F (2000). Cryopreserved primary hepatocytes as a constantly available *in vitro* model for the evaluation of human and animal drug metabolism and enzyme induction. *Drug Metab. Rev.* 32: 81-118.
- Iniaighe OM, Malomo SO, Adebayo JO (2008). Hepatoprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in carbon tetrachloride treated rats. *J. Med. Plants Res.* 2: 301-305.
- Kohen R, Nyska A. (2002). Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.* 30: 620-650.
- Lima CF, Fernandes-Ferreira M, Preira-Wilson C (2006). Phenolic compounds protect HepG2 cells from oxidative damage: relevance of glutathione levels. *Life Sci.* 79: 2056-2068.
- Runge D, Michalopoulos GK, Storm SC, Runge DM (2000). Recent advances in human hepatocyte culture systems. *Biochem Biophys Res Commun* 274: 1-3.
- Shahidi F, Naczki M (2004). *Phenolics in Food and Nutraceuticals*. CRC Press LLC, Boca Raton, Florida, USA.
- Sahu SC, Rygges DI, O'Donnell MW (2006). Prooxidant activity and toxicity of nordihydro- guaiaretic acid in clone-9 rat hepatocyte cultures. *Food Chem. Toxicol.* 44: 1751-1757.
- Starke PE, Farber JL (1985). Endogenous defenses against the cytotoxicity of hydrogen peroxide in cultured rat hepatocytes. *J. Biol. Chem.* 260: 86-92.
- Sugikara N, Arakawa T, Ohnishi M, Furuno K (1999). Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with α -linolenic acid. *Free Radical Biol. Med.* 27: 1313-1323.
- Valentová K, Ulrichová J, Cvak L, Šimánek V (2007). Cytoprotective effect of a bilberry extract against oxidative damage of rat hepatocytes. *Food Chem.* 101: 912-917.
- Vitaglione P, Morisco F, Caporaso N, Fogliano V. (2004). Dietary antioxidant compounds and liver health. *Crit. Rev. Food Sci. Nutr.* 44: 575-586.
- Wang BJ, Liu CT, Tseng CY, Wu CP, Yu ZR (2004). Hepatoprotective and antioxidant effects of *Bupleurum kanoi* Liu (Chao et Chuang) extract and its fractions fractionated using supercritical CO₂ on CCl₄-induced liver damage. *Food Chem. Toxicol.* 42: 609-617.
- Wu YH, Yang LX, Wang F, Wu XM, Zhou CX, Shi SY, Mo JX, Zhao Y (2007). Hepatoprotective and antioxidative effects of total phenolics from *Laggera pterodonta* on chemical-induced injury in primary cultured neonatal rat hepatocytes. *Food Chem. Toxicol.* 45: 1349-1355.
- Yao P, Nussler A, Liu LG, Hao LP, Song FF, Schirmeier A, Nussler N (2007). Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. *J. Hepatol.* 47: 253-261.
- Yau MH, Che CT, Liang SM, Kong YC, Fong WP (2002). An aqueous extract of *Rubus chingii* fruits protects primary rat hepatocytes against *tert*-butyl hydroperoxide induced oxidative stress. *Life Sci.* 72: 329-338.
- Zhao XH, Zhang X (2009). Comparisons of cytoprotective effects of three flavonoids on human hepatocytes oxidative injury induced by hydrogen peroxide or carbon tetrachloride *in vitro*. *J. Med. Plants Res.* 3: 776-784.

