RESEARCH ARTICLE

Pu-erh Tea Powder Preventive Effects on Cisplatin-Induced Liver Oxidative Damage in Wistar Rats

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Abstract

Background: Chemotherapy is one of the major means for control of malignancies, with cisplatin (CDDP) as one of the main agents, widely used for the treatment of various malignant solid tumors. However, prevention of hepatotoxicity from cisplatin is one of the urgent issues in cancer chemotherapy. In this study, we aimed to investigate the effects of pu-erh tea on hepatotoxicity through body weight and tissue antioxidant parameters like, liver coefficient, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), malondialdehyde(MDA) and glutathione (GSH) levels, and light microscopic evaluation by histological findings. Materials and Methods: The rats were randomly divided into five groups: Control (n=10), cisplatin (3 mg/kg p.i., n=10), cisplatin+pu-erh (0.32 g/kg/day i.g., n=10), cisplatin+pu-erh (0.8 g/kg/day i.g., n=10) and cisplatin+pu-erh (1.6 g/kg/day i.g., n=10). Pu-erh tea powder was administrated for 31 consecutive days. The rats were sacrificed at the end on the second day after a single dose of cisplatin treatment for measuring indices. Results: Pu-erh tea powder exhibited a protective effect by decreasing MDA and GSH and increasing the SOD and GSH-PX levels and GSH-PX/MDA ratio in camparison with the control group. Besides, pu-erh tea was also able to alleviate the pathological damage to some extent. Conclusion: Pu-erh tea powder is protective against cisplatin-induced liver oxidative damages, especially at the medium dosage (0.8 g/kg/d).

Keywords: Pu-erh tea powder - cisplatin - liver - oxidative damage

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Introduction

Chemotherapy is one of the major means for malignancy. It seeks to shrink tumors, decrease cancer-related symptoms, advance quality of life, lengthen life spans of patients, and so on. Many studies have shown the effectiveness and feasibility of chemotherapy for cancers species involving the lung, pancreas, liver, large intestine, and so on. Conversely, treatment with anticancer drugs can also do harm to patients' normal tissues and organs, and those toxicities may lead to a worsened quality of life and shortened survival.

Cisplatin (cis-diamminedichloroplatinumII, CDDP) is one of the main chemotherapeutics, which is widely used for the treatment of various malignant solid tumors such as ovarian, lung, testicular and head and neck cancers (Pfister et al., 2010; Dimri et al., 2013; Dua et al., 2013; Besse et al., 2014). Nevertheless, its full clinical utility is limited due to some undesirable side effects in the liver, the kidneys, and other organs. Because cisplatin is mainly metabolized through liver and kidneys in human body, nephrotoxicity and hepatotoxicity are very easy to occur. The nephrotoxicity of cisplatin is well documented as being the most important dose-limiting factor in cancer chemotherapy (Jung et al., 2014). However, cisplatin-induced hepatotoxicity has rarely been characterized, and it has been little studied (Bentli et al., 2013). The reason for this is hepatotoxicity rarely happens at standard doses, only after administration with high doses of cisplatin is it frequently observed, and can alter the clinical situation of patients (Sudhakar et al., 2010). Therefore, prevention of the side effects from cisplatin is one of the main problems in cancer chemotherapy.

Tea in China is considered a natural healthy beverage with a 3000-year history. From the perspective of traditional Chinese medicine (TCM), tea has the effects

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of clearing the mind, relieving restlessness, benefiting the thinking ability, improving acuity of vision, detoxifying, promoting urine discharge, quenching thirst, relieving cough, resolving phlegm, helping digestion, and so on. During the last three centuries, many specialists and scholars have been researching more extensively and deeply the efficacy and low toxicity of natural health beverage-tea. It has been proved that tea has the properties against aging, angiocardiopathy, cancer, radiation injury, viruses, bacteria and so on. Antioxidation is thought to be one of the most important mechanisms of tea's health care functions. Currently, there are many reports and studies on antioxidation and the mechanisms of green tea (Pandurangan et al., 2012; Cai et al., 2013; Haidari et al., 2013), while seldom of pu-erh tea (Wang et al., 2012).

Recently evidence has been accumulated that those side effects like nephrotoxicity and hepatotoxicity are closely related to the activity of reactive oxygen species (ROS) (Gurocak et al., 2013; Rodrigues et al., 2014; Wang et al., 2014). ROS including superoxide anion radical (O_2·-), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and nitric oxide (NO), which are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins and nucleic acids in tissues. CDDP causes the generation of ROS, depletion of GSH, and inhibition of antioxidant enzymes activity. Johns et al. (2012) concluded that cisplatin therapy had been shown to induce high levels of lipid peroxidation in lung cancer patients and could be assessed from the 8-isoprostane marker in overnight urine, with or without volume correction (Johns et al., 2012). Thus, the administration of antioxidants such as vitamin C, vitamin E, Silymarin, selenium, and so on before the administration of CDDP has been used to protect against kidney and liver toxicities (Ajith et al., 2009; Abdelmeguid et al., 2010; Ghorbani et al., 2013).

The aim of this study was to investigate possible protective effects of pu-erh tea on CDDP-induced oxidative liver injury, and its effects on the levels of body weight, liver coefficient, ALT and AST, SOD and GSH-PX, MDA and GSH, as well as histological changes in a rat model.

Materials and Methods

Chemicals, reagents and instruments

Pu-erh tea powder was kindly provided by Prof. Shengjun from Yunnan Agricultural University (1 gram of tea powder is equivalent to 10 grams of tea water extracts). Cisplatin was purchased from Qilu Pharmaceutical co., Ltd. (Ji'nan, China). MDA, SOD, GSH and GSH-PX kits were purchased from Nanjing KeyGen Biotech co., Ltd. (Nanjing, China). Instruments are a ultraviolet and visible spectrophotometer (UNICO WEZUV-2000, USA), a centrifuge (HITACHI, CF16RX, Japan), some tissue grinders (Dounce, USA), a light microscope (OlympusIX51, Japan).

Animals and treatments

50 adult male Wistar albino rats, 200±20g, 6-week old, were used in this study. They were provided by Laboratory Animal Center of Tsinghua University, Beijing, China. The animals were housed in metal cages under a standard room ambient temperature, and 12:12 hours light/dark cycles with free commercial standard rat chow and water ad libitum during the whole experimental process (rat chow given except the last 2 days of the experiment). All animals were housed for one week of acclimatization before the experiment. This research was approved by Animal Ethics Committee of Tsinghua University (No.2010-TangJT-Tea-2), and following the "Guide for the care and Use of Laboratory Animals, DHEW Publication No. (NIH) 85-23, 1985".

The rats were randomly divided into five groups, each with ten rats, and were named according to their experimental treatments: (1) the control: (*p*.i.); (2) the cisplatin: (*p*.i.) 3 mg/kg cisplatin (Cisplatinum Ebewe, 0.5mg/ml physiological saline) injected on the 30th day of the experimental period at a time; (3) the cisplatin+0.32 g/kg/day pu-erh tea powder: Cisplatin the same, 0.32 g/kg/day pu-erh tea powder (i.g.) given all 31 days of the experimental period; (4) the cisplatin+0.8 g/kg/day pu-erh tea powder: Cisplatin the same, 0.8 g/kg/day pu-erh tea powder (i.g.) given all 31 days of the experimental period; (5) the cisplatin+1.6 g/kg/day puerh tea powder: Cisplatin the same, 1.6g/kg/day pu-erh tea powder (i.g.) given all 31 days of the experimental period. The rats were sacrificed at the second day of cisplatin treatment. All animals were anaesthetized through inhalation of ether, and then sacrificed by cervical dislocation 24 hours after the final saline and CDDP injections. Then, the blood was collected by picking the eyeballs for serum parameters. The livers of the rats were removed, and washed with physiological saline solution, pieced into two for biochemical and light microscopic examinations respectively, and the part used as biochemical examination were stored at -80°C until analysis.

Liver coefficient

Livers were removed and weighed right after collecting blood. And then liver coefficients was calculated. (liver coefficient = liver wet weight/body weight×100%).

Histopathological examination

Liver biopsies were fixed in 10% neutral buffered formalin, embedded in paraffin wax and cut sections were stained using haematoxylin and eosin (HE). Histopathological examination was performanced by Institute of Clinical Medical Sciences in China-Japan Friendship Hospital (Beijing, China).

Serum biochemical analysis

ALT and AST were determined by Hitachi 7076 autobiochemistry analyzer. Serum biochemical analysis was carried out by Tsinghua University Hospital.

Determination of liver MDA, SOD, GSH and GSH-PX

All tissues were maintained at 4°C throughout preparation. A portion of liver tissues for all the assays were homogenized in a 0.9% NaCl solution (NaCl/liver tissue, 9:1, v/v). Tissue homogenates were centrifuged for 15 minutes at 15, 000 g, and then the clear upper

Table 1. Influence of Various Doses of pu-erh Tea Powder on Body Weight and Liver Coeffficient

Group	n	Body weight (g)	Liver coefficient (%)
Control	10	328.3±17.88	2.89±0.31**
Cisplatin (3 mg/kg)	10	300.3±25.16	3.79±0.38b
Cisplatin+Pu-erh (0.32 g/kg/d)	10	318.5±29.84	3.44±0.44b*
Cisplatin+Pu-erh (0.8 g/kg/d)	10	318.4±29.57	3.24±0.47a**
Cisplatin+Pu-erh (1.6 g/kg/d)	10	322.4±23.06	3.03±0.23**

Data represent mean ±SD; n=10; [a]p<0.05, [b]p<0.01, compared to the control group; *p<0.05, **p<0.01, compared to the cisplatin group

supernatants were removed for analysis. Analysis of the samples was carried out after the end of the experiment. MDA, SOD, GSH and GSH-PX were determined by the appropriate test kits using a spectrophotometer.

Statistical analysis

Data are presented as mean ±SD. All statistical analyses were performed using the statistical software package for the social sciences for windows (SPSS 11.0). The one-way ANOVA analysis of variance and post hoc multiple comparison tests (LSD) were carried out to examine the differences among groups. Statistical significance was defined as p<0.05.

Results

Changes in liver coefficient

The liver coefficient results are expressed in Table 1. There was no statistical significance in body weights among the five groups of rats after 31 days (p>0.05). The liver coefficients of cisplatin group, cisplatin+pu-erh (0.32 g/kg/d) group and cisplatin+pu-erh (0.8 g/kg/d) group were significantly increased in comparison with the control group (p<0.05). Compared to the cisplatin group, all the other groups' liver coefficients were significantly decreased (p<0.05).

Changes in histopathological examination

The histological changes were evaluated and are presented in Figure 1. The livers of the control animals showed normal histology (A). In the liver sections of the cisplatin group, sinusoidal congestion, hepatocellular edema, degeneration and necrosis, and inflammatory cell infiltration were observed (B, C, D, E and F). While in the cisplatin+pu-erh groups, marked decreases in cytoplasmic changes of the hepatocytes, sinusoidal congestion, hepatocellular edema, degeneration and necrosis, and inflammatory cell infiltration were noticed when compared to th cisplatin group (G, H and I). Of all the three cisplatin+pu-erh groups, changes in the cisplatin+pu-erh (0.8 g/kg/d) group was most obvious in comparison to the other two groups.

Changes in serum biochemical analysis

As shown in Table 2, one day after the intraperitoneal injection of ciplatin on the 31th day of this experiment, rats' serum transaminases ALT and AST in the cisplatin, cisplatin+pu-erh (0.32 g/kg/d), cisplatin+pu-erh (0.8 g/kg/d) and cisplatin+pu-erh (1.6 g/kg/d) groups were significantly increased in comparison with the control group (p<0.01). But all the pu-erh groups' ALT and AST (except the ALT of cisplatin+pu-erh (1.6 g/kg/d) group was more higher than the cisplatin group) were no significant different with the cisplatin group (p>0.05).

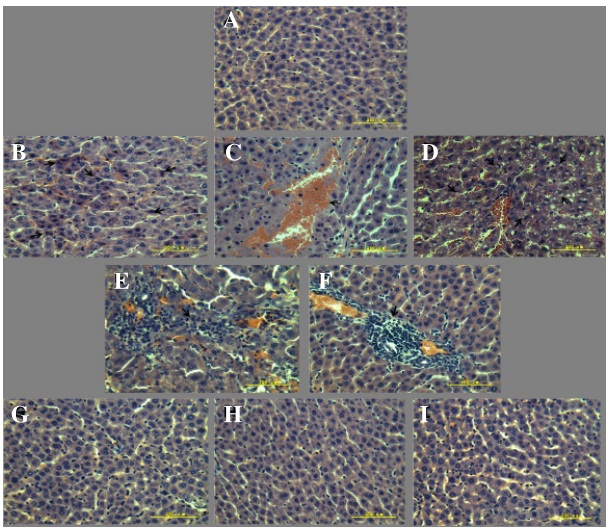

Figure 1. Representive Light Micrographs in The Liver Tissues of Rats in different Groups. A: Control group; **B**, **C**, **D**, **E** and **F**: Cisplatin group; **G**: Cisplatin+ pu-erh (0.32 g/kg/d) group; **H**: Cisplatin+ pu-erh (0.8g/ kg/d) group; **I**: Cisplatin+ pu-erh (1.6 g/kg/d) group. Arrows show sinusoidal congestion, hepatocellular edema, degeneration and necrosis, and inflammatory cell infiltration in cisplatin group

Table 2. Influence of Various doses of pu-erh Tea Powder on ALT and AST

Group	n	ALT (IU/L)	AST (IU/L)
Control	10	37.40±10.40**	105.70±24.59**
Cisplatin (3 mg/kg)	10	61.80±10.95[b]	232.50±41.19[b]
Cisplatin+Pu-erh (0.32 g/kg/d)	10	59.90±8.90[b]	220.70±37.21[b]
Cisplatin+Pu-erh (0.8 g/kg/d)	10	57.00±8.37[b]	201.50±21.01[b]
Cisplatin+Pu-erh (1.6 g/kg/d)	10	71.10±7.13[b]*	238.30±43.13[b]

Data represent mean ±SD; n=10; [a]p<0.05, [b]p<0.01, compared to the control group; *p<0.05, **p<0.01, compared to the cisplatin group. ALT's normal reference range is 17.5~52.0 IU/L; AST's normal reference range is 45.7~200.0 IU/L (Li C, 2008).

Changes in liver MDA, SOD, GSH, GSH-PX and GSH-PX/MDA

The effect of pu-erh tea on cisplatin-induced oxidative stress are demonstrated in Table 3. The MDA level in the cisplatin-related groups was significantly higher than in the control group (p<0.01). While the GSH levels, GSH-PX activities and GSH/MDA values were significantly lower than in the control group (p<0.01). There was a significant difference in MDA levels between the cisplatin and the other groups (except the cisplatin+pu-erh (0.32 g/kg/d) group) (p>0.05). Similarly, the changes of GSH levels, GSH-PX activities and GSH/MDA values were like the MDA in this experiment. The activities of SOD in the cisplatin, cisplatin+pu-erh (0.32 g/kg/d) and cisplatin+pu-

Table 3. Influence of Various doses of pu-erh Tea Powder on MDA, SOD, GSH, GSH-PX and GSH-PX/MDA

Group	n	MD (nmol/mg prot.)	SOD (U/mg prot.)	GSH (mg/g prot.)	GSH-PX (U/g prot.)	GSH-PX/MDA (%)
Control	10	6.71±0.70**	61.36±6.94**	50.28±2.12**	173.37±5.17**	26.10±2.99**
Cisplatin (3 mg/kg)	10	9.63±0.66[b]	41.07±3.97[b]	38.83±2.41[b]	98.82±8.90[b]	10.28±0.96[b]
Cisplatin+Pu-erh (0.32 g/kg/d)	10	9.16±0.93[b]	43.71±5.15[b]	40.31±2.95[b]	103.32±10.41[b]	11.38±1.50[b]
Cisplatin+Pu-erh (0.8 g/kg/d)	10	8.68±0.57[b]**	61.14±4.92**	42.37±3.62[b]**	120.83±13.79[b]**	13.97±1.86[b]**
Cisplatin+Pu-erh (1.6 g/kg/d)	10	8.63±0.74[b]**	42.12±4.60[b]	46.03±2.97[b]**	116.97±10.41[b]**	13.67±1.85[b]**

Data represent mean ±SD; n=10; [a]p<0.05, [b]p<0.01, compared to the control group; *p<0.05, **p<0.01, compared to the cisplatin group

erh (1.6 g/kg/d) groups were significantly decreased in comparison with the control group (p<0.01). Compared to the cisplatin group, the SOD activity in cisplatin+pu-erh (0.8 g/kg/d) group was significantly increased (p<0.01). There were no significant difference between the cisplatin, cisplatin+pu-erh (0.32 g/kg/d) and cisplatin+pu-erh (1.6 g/kg/d) groups (p>0.05).

Discussion

Cisplatin is one of the most active cytotoxic agents against cancer. Hepatotoxicity is one of its side effects. In this experiment, cisplatin's influences on anti-lipid-perioxidation parameters of rats' livers indicated that cisplatin can cause the obvious oxidative damage of liver. That's to say, one of the mechanisms about cisplatin-induced liver injury is oxidative damage. Naqshbandi A et al (2012) showed that dietary supplementation of flaxseed oil in cisplatin-treated rats ameliorated cisplatin-induced hepatotoxic and other deleterious effects due to its intrinsic biochemical/antioxidant properties (Naqshbandi A et al., 2012). Gaona-Gaona L et al (2011) investigated the protective effect of sulforaphane pretreatment against cisplatin-induced liver and mitochondrial oxidant damage in rats. They proved that the hepatoprotective effect of sulforaphane was associated to the preservation of mitochondrial function, antioxidant enzymes and prevention of liver and mitochondrial oxidant stress (Gaona-Gaona L et al., 2011).

A Chinese ancient TCM book named Ben Cao Zai Xin says pu-erh tea can "enter into the liver and stomach channels" and "clear away the pathogenic heat of liver and gallbladder". So presumably, from TCM's point of view, pu-erh tea enters into the liver channel so that it can treat liver diseases. A modern research done by Wang D et al (2011) has shown that the DNA damage, as well as the reactive oxygen species (ROS), was decreased, while the activity of antioxidative system was increased in mice liver after co-administration of Quinocetone and Pu-erh black tea extract (Wang D et al., 2011).

ALT mainly exists in the liver cell sap and mitochondria, but not in blood serum. Since the enzymes' activities of liver is about a thousand times as high as of serum, if only one percent of hepatocytes in the liver necrotize, then the enzymes' activities of serum will double. Thus, ALT becomes one of the most sensitive parameters for liver function test as recommanded by WHO. Any reason induced liver cell injury can cause a spike in serum ALT. AST is higher in the cardiac muscle, then in the liver. It has two isoenzymes, ASTs and ASTm respectively. In the normal serum mainly is ASTs. When there comes necrosis of a chunk of liver tissue, ASTm is released from in the liver mitochondria, so that AST in the blood serum increases obviously. Since the serum ALT and AST can better reflect the extent of liver damage, they are the key indexes measuring the level of liver injury. Zhao JA et al (2014) demonstrated that diethylnitrosamine (DEN) induced severe histological and immunohistochemical changes in liver tissues, significantly increasing the levels of liver marker enzymes such as ALT and AST. Taken together, their results suggested application of curcumin (CUR) and hydrazinocurcumin (HZC) could prevent the occurrence of carcinogenesis and HZC may be a more potent compound for prevention of DEN-induced hepatocarcinogenesis in rats than CUR (Zhao JA et al., 2014). Duh PD et al (2010) investigated the in vitro and in vivo protective effects of water extract of pu-erh tea (WEPT) on tert-butyl-hydroperoxide (t-BHP)-induced oxidative damage in hepatocytes of HepG2 cells and in rat livers. They found that the administration of WEPT before a single dose of t-BHP exhibited a significant protective effect by lowering serum levels of ALT and AST (Duh PD et al., 2010).

In our experiment, except the ALT of cisplatin+pu-erh (1.6 g/kg/d) group was even more higher than the cisplatin group, all the other pu-erh groups' ALT and AST had a downward trend (the most obvious group was cisplatin+pu-erh (0.8 g/kg/d)), but there was no significant difference with the cisplatin group (p>0.05). Therefore, ALT and AST alone are still difficult to determine the functions of pu-erh tea. Garcia-Cortes M et al (2008) analyzed the demographics, and clinical and epidemiological characteristics of patients developing liver injury related to the natural remedies. They found that the predominating type of liver damage was hepatocellular (12.92%) and 31% of all thirteen cases exhibited the common features of hypersensitivity. Camellia sinesis (3.23%) was the main causative herb (Garcia-Cortes M et al., 2008). So, as a result, the reason for the increment of ALT and AST values in the cisplatin+pu-erh (1.6 g/kg/d) group compared with the cisplatin group still need to be confirmed.

SOD are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen. GSH-PX is the general name of an enzyme family with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of GSH-PX is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water. GSH is a tripeptide that contains an unusual peptide

linkage between the amine group of cysteine (which is attached by normal peptide linkage to a glycine) and the carboxyl group of the glutamate side-chain. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. MDA is the organic compound with the formula $CH_2(CHO)_2$. The structure of it is more complex than the formula itself suggests. These reactive species are produced naturally and are markers for oxidative stress. Results of a study indicated oxidative stress and DNA damage activity increased in multiple myeloma patients and were alleviated in response to therapy. Relative indicators like SOD, MDA, GSH-PX and so on were analyzed in sixty patients before and after one month treatment with induction therapy (Mehdi WA et al., 2013).

In Fan JP et al (2013)'s study, they evaluated the free radical scavenging and anti-oxidative activities of an ethanol-soluble pigment extract prepared from fermented Zijuan Pu-erh tea. Their final results suggested that the extract has excellent free radical scavenging and anti-oxidative properties by increasing the activity of SOD in cells and decreasing the concentration of MDA (Fan JP et al., 2013). In a study on pu-erh tea aqueous extracts lowering atherosclerotic risk factors in a rat hyperlipidemia model, Hou Y et al (2009) confirmed that compared to the hyperlipidmic control group, activities of SOD and GSH-PX in serum were significantly elevated in pu-erh tea-treated groups while levels of MDA decreased in the same groups. These results indicated that pu-erh tea exerts a strong antioxidative effect (Hou Y et al., 2009).

In our study, the GSH levels, GSH-PX and SOD activities were significantly lower than in the control group ($p<0.01$), and the MDA level in the cisplatin-related groups was significantly higher than in the control group ($p<0.01$). These results showed that cisplatin decreases the antioxidant power, so that it increases the peroxidation damages of the body, whose results were fairly in accorded with some studies' (Sahu BD et al., 2011; Buldak RJ et al., 2012; Gupta RK et al., 2013; Zhao YM et al., 2014). In this experiment, the oxidative stress indices in the pu-erh tea related groups changed to some extent compared to the cisplatin group, including the activities of SOD and GSH-PX and the levels of GSH were increased, while the levels of MDA were decreased. Of the four pu-erh tea related groups, all four parameters in the cisplatin+pu-erh (0.8 g/kg/d) tea group were significantly changed ($p<0.01$); three parameters (SOD not included) in the cisplatin+pu-erh (1.6 g/kg/d) were significantly changed ($p<0.01$). These experimental results proved of pu-erh tea's significant antioxidative activities.

It is thought that when we evaluate the antioxidation of drugs, it is supposed that GSH-PX activity or MDA level as assessment indicators alone is not enough, which means that, moreover, we should combine the two as GSH-PX/MDA, reflecting the potential antioxidant activities in the organs and cells of human body and fully evaluating the type of antioxidation (Chen Y and Zhou M, 1991). In a study about the effects of tea polyphenols (TP) on microcirculation and antioxidation in aircrew, Luo XM et al (1999) found out the proper antioxidant for the health protection of aircrew. The results showed that GSH-PX (whole blood GSH-PX)/MDA (serum MDA) ratio in TP group were significantly higher than those in control group (Luo XM et al., 1999). Besides, Wang Z et al (2011) and Fabian E et al (2011) also applied the GSH-PX/MDA ratio in their experiments, which indicated the importance of this index (Wang Z et al., 2011; Fabian E et al., 2011).

To make sure the antioxidative function of pu-erh tea, our experiment opted for the GSH-PX/MDA ratio. The changes of GSH-PX/MDA ratio in the experiment showed that cisplatin can decrease the potential antioxidative capacity of rats' liver cell, while pu-erh tea was able to reverse this condition.

Some other researches have already proved that cisplatin can cause pathological changes in liver tissue (Nasr AY, 2013; Palipoch S et al., 2014). Similarly, in our study, the animals' liver tissue in the cisplatin group showed rather obvious pathological changes. While pu-erh tea was able to alleviate the pathological damages to some extent, so that we can say pu-erh tea is able to prevent rat liver from cisplatin-induced oxidative damages. By contrast, the antioxidative effect of cisplatin+pu-erh (0.8 g/kg/d) group was most obvious.

In the present study, we demonstrated the protective role of pu-erh tea powder on the cisplatin-induced liver oxidative damages in rats, especially pu-erh tea powder of the medium dosage (0.8 g/kg/d). Moreover, our study results also showed that serum ALT and AST in the cisplatin+pu-erh (1.6 g/kg/d) group were increased in comparison with the cisplatin group. It may be because of high concentration pu-erh tea itself, or experimental errors, or lacking in samples. Therefore, further repeated experiments are necessery to make sure the results.

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