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Puerarin protects rat liver and kidney against cadmium-induced oxidative stress

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ABSTRACT

Oxidative stress is thought to be involved in cadmium (Cd) induced toxicity. This study examined the possible protective effect of puerarin on cadmium chloride ($CdCl_2$, 2 mg/kg b.w.) induced toxicity in male rats. Male SD rats were treated with either intraperitoneal Cd and/or oral puerarin (100 mg/kg. b.w.) for 4 weeks. The results demonstrated that exposure to Cd led to an increase in the level of BUN, ALT and AST in serum. Cadmium raised the concentrations of MDA and GSH, and decreased antioxidants activities (SOD, CAT, and GSH-Px) in the liver and kidney. Conversely, administration of puerarin markedly attenuated Cd-induced biochemical alterations in serum, liver, and kidney tissues. These results suggest that puerarin exerts protective effects against Cd toxicity attributable to its antioxidant actions.

Key words: Cadmium, Kidney, Liver, Oxidative stress, Puerarin

Cadmium (Cd) is a persistent bioaccumulating element in the environment (Nwokocha *et al.* 2012). Together with other heavy metals, Cd was listed in the International Register of Potentially Toxic Chemicals by the United Nations Environment Program (Nna *et al.* 2017). It has been listed as one of the top ten toxic compounds for human health (Hirano *et al.* 1996). Because of good characters, Cd was widely used in industries for rubber processing, galvanizing, nickel and Cd batteries in the electrode material, plastic and glass pigments, pesticides production and poly vinylchloride (PVC) stabilizer and other industries (Kidambi *et al.* 2003).

Cadmium is present in air, soil, water and food (Calderon *et al.* 2003, Satarug *et al.* 2004, Sarkar *et al.* 2013). People are exposed to Cd from water, rice, potato, tobacco, soil, sleek seeds, root and leaf of vegetables which were polluted by Cd. Exposure to exogenous chemicals can lead to the production of free radicals, which can result in oxidative stress when the free radical production exceeds the protective effect of antioxidants (Wu *et al.* 2003).

It is reported that Cd and several other industrial chemicals can cause oxidative stress in different cells and organs of the body (Patra *et al.* 1999; Valko *et al.* 2005). The toxicity of Cd has been reported in liver, kidney, lungs, brain, bones, testes and other organs. Cadmium can cause cancer and histopathological damage to male reproductive organs (Massanyi *et al.* 2007; Wang *et al.* 2018).

Pueraria root has become widely used in the Western dietary supplements recently, and it is a traditional chinese medicine (Prasain *et al*. 2012). Puerarin is a major isoflavone compound separated from the root of *Pueraria lobata*. Researchers have shown that puerarin has the abilities of antioxidative, anti-apoptosis, renal protection and hepatoprotective effects (Liu *et al.* 2011, 2012, Xie *et al.* 2011). Despite the pharmacological benefits, the mechanisms of the protective effects of puerarin are still unclear. The aim of this work was to investigate the conceivable ameliorative impact of puerarin on Cd induced toxicity on SD rats.

MATERIALS AND METHODS

Male SD (Sprague Dawley) rats (6 weeks old, 180–200 g) were purchased from Experimental Animal Center (Zhengzhou, China). The experimental animals were free to obtain rodent food under the conditions of constant temperature (24±2°C, with air conditioner) under 12 h light/dark schedules in animal house of Henan University of Science and Technology. These animal investigations were carried out in accordance with the University Animal Care and User Committee guidelines at Henan University of Science and Technology.

Chemicals and reagents: Reagents and kits used in the assays were of the blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), reduced glutathione (GSH), maleic dialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) from Nanjing Jiancheng (Nanjing, China). $CdCl_2$ was purchased from Aladdin Industrial

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Corporation. Puerarin (>98% purity) was purchased from Mianyang Oriental Source Technology Co., Ltd (Mianyang, China). All other reagents were analysis grade.

Experimental design: After acclimatization to the laboratory conditions for 2 weeks, the rats were randomly divided into four experimental groups with 6 rats in each group. They were treated everyday as follows: control group; $CdCl_2$-treated group (received 2 mg/kg bw of $CdCl_2$ intraperitoneally); puerarin-treated group (received 100 mg/kg bw of puerarin by oral gavage); and $CdCl_2$ + puerarin-treated group (received 2 mg/kg bw of $CdCl_2$ intraperitoneally and 100 mg/kg bw of puerarin intragastrically).

The experiment lasted for 4 weeks. Rats were weighed every week. 24 h after the administration of the last dose, the rats were anesthetized with ether anesthesia and sacrificed by decapitation.

Blood and tissue sample preparation: Blood samples were collected for hematological studies in EDTA test tubes. The second blood samples were collected from femoral artery into tubes from all the animals in each group for serum separation. The liver and kidney were removed, then weighed. The relative weight of the livers and kidneys were calculated.

Hematological studies: The determination of complete blood count, red blood cell counts (RBC), mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume (MCV), platelet (PLT) counts, and mean platelet volume (MPV) were assessed according to the standard hematological techniques.

Biochemical assays: The levels of ALT, AST, BUN were measured in serum using commercial kits.

Liver and kidney were kept at –80°C. They were homogenized in PBS (pH 7.4), then centrifuged (3,000 g, 10 min) before analysis. The resultant supernatant was utilized for analysis of MDA, GSH, SOD, CAT, and GSH-Px activities using commercial kits.

Statistical analyses: The results were represented by the mean±SE of number of observations. All statistical analyses were carried out by SPSS 15.0 statistical software. Comparison of means was carried out by one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). Values were considered significant when $P<0.05$ or $P<0.01$.

RESULTS AND DISCUSSION

Changes in the body weight, relative weight of organ: No significant changes were observed between puerarin alone treated and control group (Table 1). In Cd-treated rats, the body weight gain reduced significantly compared with the control group ($P<0.01$). Treatment with puerarin (100 mg/kg) alongside cadmium increased the body weight compared with the cadmium group. However, there was no significant difference between them ($P>0.05$). Weight gain is related to the availability and absorption of nutrients (Asagba and Eriyamremu 2007). The Cd-induced decrease of nutrient digestion and absorption have also been reseached (Eriyamremu *et al*. 2005). Cadmium inhibits the action of digestive and absorption enzymes (Asagba 2010). This finding may explain the weight loss of rats as observed in this experiment.

At the same time, the relative weight of liver increased significantly ($P<0.01$). However, The group which was treated with puerarin (100 mg/kg) alongside cadmium showed that the decrease of relative weight of liver was significant compared with the control group ($P<0.05$) after the exposure for 4 weeks.The alterations of the relative weight of organ in Cd intoxicated rats could be due to tissue damage and the reduction in their functions (Pari and Shagirtha 2010). But cadmium can increase the relative weight of kidney, however, compared with the cadmium group, there was non-significant difference ($P>0.05$).

Table 1. Body weight, relative weight of organ in control and experimental rats

Group	Body weight		Relative weight of organ	
	Initial (g)	Final (g)	Liver	Kindey
Control	176.92±7.61	274.37±4.97	3.12±0.13	0.82±0.03
Cadmium	172.40±4.55	232.50±9.65**	3.67±0.19**	0.95±0.05
Puerarin	175.38±4.96	280.72±7.61	3.17±0.18	0.84±0.04
Cadmium +puerarin	177.14±5.61	240.57±7.54	3.46±0.15$^{\#}$	0.87±0.02

*$P<0.05$, **$P<0.01$ (compared with control group). $^{\#}P<0.05$, $^{\#\#}P<0.01$ (compared with Cd exposed group).

Hematological parameters: Cadmium-exposed group showed a significant decrease ($P<0.05$ or $P<0.01$) in RBC, MCHC, Hb and HCT in relation to the control rats. In addition, MCV, PLTs, MPV were non-significant changed in comparison with the control group. Cadmium and puerarin exposure increased the RBC, MCHC, Hb and HCT when compared with Cd treated rats significantly ($P<0.05$ or $P<0.01$) (Table 2).

Exposure to Cd can result in toxic effects on blood, because Cd binds to the erythrocyte membrane, causing not only qualitative and quantitative leukocyte diseases, but also hematological disturbance such as anemia (Yuan *et al.* 2014). In the current study, Cd exposure caused anemia. Anemia in rats after treatment with Cd was observed in several other studies (Ashour 2014, Mladenoviæ *et al.* 2014). Accumulation of Cd in hematopoietic tissues, liver, kidney, and spleen might inhibit erythropoietic activity (Horiguchi *et al.* 2011). In addition, Cd causes anemia by accelerating the rate of erythrocyte destruction (hemolysis) due to changes in membrane permeability caused by kidney damage (Ashour 2014, Yuan *et al.* 2014). Moreover, inflammatory cytokines play an important role in inhibiting erythropoiesis of peripheral blood cells (Dakeshita *et al.* 2009).

Cadmium-induced dysfunction of liver and kidney and the protective effect of puerarin: Previous studies showed

Table 2. Effect of Cd and puerarin on the blood physiological parameters in rats

Group	Control	Cadmium	Puerarin	Cadmium + puerarin
RBC ($\times10^{12}$/L)	7.60±0.45	5.46±0.77*	7.35±0.39	6.01±0.33#
MCHC (g/L)	297.67± 9.63	269.17± 14.08*	300.23± 1.86	301.82± 1.40##
Hb (g/L)	138.67± 10.96	88.17± 11.36**	130.43± 7.98	111.41± 5.33##
HCT (%)	46.42± 3.05	31.13± 4.28**	43.34± 2.56	38.82± 1.62#
MCV (fL)	61.20±1.67	60.05±1.13	58.98±1.53	61.56±1.17
PLT ($\times10^9$/L)	590.50± 11.63	581.33± 12.94	620.82± 39.81	607.34± 53.66
MPV (fL)	7.72±0.21	7.75±0.13	7.34±0.07	7.68±0.09

*P<0.05, **P<0.01 (compared with control group). #P<0.05, ##P<0.01 (compared with Cd exposed group).

that exposure to Cd leads to liver damage and dysfunction (El-Boshy *et al.* 2017). The results of this study showed that the activities of ALT and AST in the serum of Cd-treated rat were significantly increased (P<0.01, Fig. 1) which is in agreement with previous studies (Cao et al. 2017). These elevated enzymes indicate that cell overflow and destruction of liver membrane structure and thereby function get affected. The administration of puerarin (100 mg/kg) significantly decreased Cd-induced hepatotoxicity as shown by diminished ALT and AST levels (P<0.05 or P<0.01). Puerarin has a strong protective effect and is widely used in clinical practice for the treatment of liver disease (Hwang *et al.* 2007, Liu *et al.* 2011, Li *et al.* 2013). The protective effect of puerarin on liver damage by Cd in rats may be achieved by reducing the activities of serum ALT and AST. This study indicates that puerarin has protective effects on liver damage induced by Cd in rats. This defensive impact of puerarin might be because of its action of restraining the Cd induced generation of ROS and keep up the structural integrity of the membrane.

Kidney damage due to Cd intoxication could be evaluated by measuring the serum markers of renal functional integrity, which are the biochemical signs of renal tissue damage. BUN is utilized for evaluating renal glomerular function filtration and its concentrations in the serum depends generally on glomerular function. In this study, the serum BUN level in Cd-treated group was higher than the control rats (P<0.01) (Fig.1). Significant restoration of BUN was observed in the rats concurrently treated with puerarin, offering protection against Cd toxicity. This finding shows that the administration of Cd modified the glomeruli and tubular capacity. A comparable finding was made by Gaurav et al. (2011), who reported that BUN levels were expanded in the serum of Cd-intoxicated rats. However, this finding is not in concurrence with Horiguchi et al. (1996), who observed that administration of Cd did not modify the BUN level in rats. The absence of consistency is likely owing to the course of introduction and dose utilized in their study.

Contents of GSH and MDA in the liver and kidney: GSH and MDA increased significantly (P<0.05 or P<0.01) in Cd treated groups compared to the control group (Fig. 2). However, compared to $CdCl_2$ group, GSH and MDA concentrations were significantly decreased (P<0.05 or P<0.01) in Cd + puerarin groups. GSH is a tripeptide and a cysteine rich protein that participates in the maintenance of cytoplasmic and membrane thiol status. It is an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS. In the current study, Cd increases the contents of GSH in liver and kidney. It could be because of increased use of GSH by the cells act as scavengers of free radicals induced by Cd. Researches have demonstrated that Cd presentation is connected with

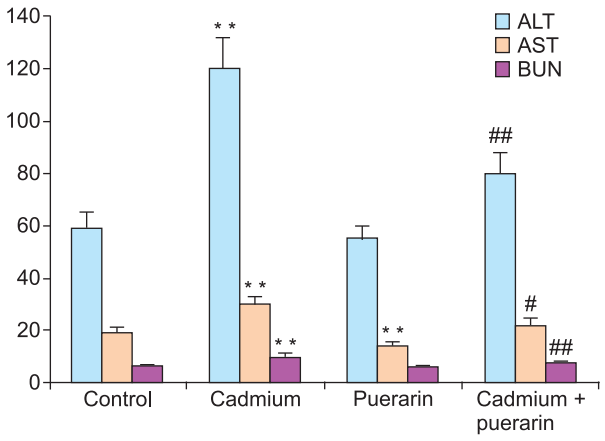

Fig. 1. Effect of puerarin on changes of ALT (U/L), AST (U/L) and BUN(mmol/L) in serum induced by Cd in rats. (*P<0.05, **P<0.01 compared with control group, #P<0.05, ##P<0.01 compared with Cd-exposed group).

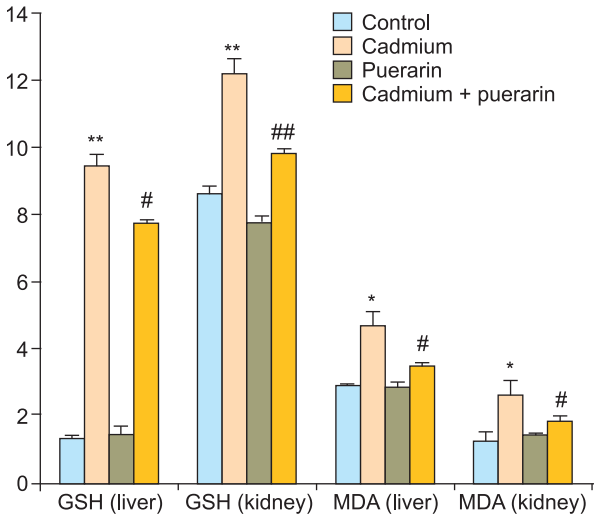

Fig. 2. Effects of Cd and puerarin on GSH (mg/g prot) and MDA (nmol/mg prot) contents in rats liver and kidney. (*P<0.05, **P<0.01 compared with control group, #P<0.05, ##P<0.01 compared with Cd-exposed group).

oxidative stress by creating ROS and LP (Xu *et al.* 2010). LP inactivates cellular components by oxidative stress in free radical chain reaction, at last resulting in loss of membrane integrity or oxidation. In this study, increased MDA level due to Cd exposure induce excessive free radicals formation which do harm to biological macromolecules (Stohs *et al.* 2001). Cd intoxicated rats pretreated with puerarin demonstrated a marked decrease in the levels of GSH and MDA. This might be because of puerarini, a good free radicals scavenger, which hinders lipid peroxidation and protein carbonylation.

*Antioxi*dant activities in the liver and kidney: Our results showed that the activities of antioxidative enzymes SOD, CAT and GSH-Px decreased in the liver and kidney of the rats exposed to Cd (Fig.3, $P<0.05$ or $P<0.01$). It was on the contrary with that of testes (data not shown). Our results are consistent with Djuric *et al.* 2015, who reported that SOD and CAT decreased in Cd-treated male Wistar rats for 21 days. SOD, CAT and GSH-Px are strong antioxidants that defend the body against oxidative stress. SOD catalyzes the removal of superoxide radicals from cells, while CAT and GSH-Px are involved in elimination of H_2O_2. In order to clarify the mechanisms of puerarin exerted effect on Cd-induced liver and kidney toxicity, we treated rats with puerarin and Cd. The activities of SOD, CAT and GSH-Px were increased significantly ($P<0.05$) in Cd + puerarin groups compared to Cd group. We found that puerarin decreased the activities of SOD, CAT and GSH-Px significantly. It showed that puerarin could reduce oxidative injury induced by Cd in rats.

In conclusion, our results showed that Cd was able to cause marked oxidative stress in addition to depletion of the antioxidants and inhibits the activities of antioxidant enzymes. Puerarin has effective protective effects on Cd-induced oxidative stress in rat liver and kidney. The results showed that puerarin seems to be potent hepatoprotective and nephroprotective drug to maintain a healthy liver and kidney.

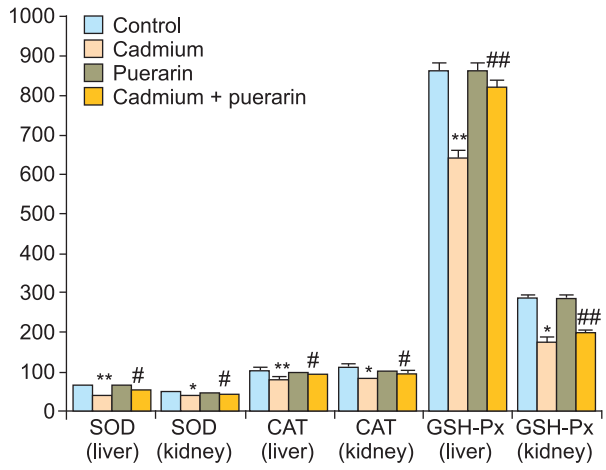

Fig. 3. Effect of Cd and puerarin on SOD (U/mgprot), CAT (U/mgprot), GSH-Px(U/mgprot) levels of rats liver and kidney (*$P<0.05$, **$P<0.01$ compared with control group, $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared with Cd-exposed group).

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