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Effects and mechanism of low molecular weight fucoidan in mitigating the peroxidative and renal damage induced by adenine

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ABSTRACT

In the present study, the beneficial effects of two sulfated polysaccharides DF and UF on adenine-induced chronic kidney disease (CKD) were investigated. Compare the chemical composition of DF and UF, DF had more sulfated group than UF, however, UF had more uronic acid. The results showed DF and UF had significant protective role in deleting the peroxidative and renal damage in CKD rats. Both samples exhibited nearly the same effect on the SUN and SCR level, however, DF showed greater effect on activity/level of CAT, GSH-PX, GSH and MDA in serum and liver compared to UF. That was to say the sample with higher content of sulfate group exhibited greater antioxidant activity. There was a positive correlation between MDA level and renal membrane damage indicating a striking relation between free radical formation and cellular injury. The mechanism of DF and UF on the CKD rats had relationship with their antioxidant activities, the samples which could enhance the activity of antioxidant enzymes and reduce the LPO level could alleviate the symptom of CKD complications.

1. Introduction

Adenine-induced CKD model provides valuable information about the pathomechanism of various complications associated with a persistent uremic state. Long-term feeding of adenine to rats produced metabolic abnormalities resembling CKD complications in humans, it could increase serum uric acid, creatinine and urea nitrogen by decreasing the urinary excretion of uric acid, creatinine and urea (Yokozawa, Kanai, & Oura, 1977; Yokozawa, Zheng, Oura, & Koizumi, 1986). Exposure to a high concentration of adenine results in the production of free radicals, which induces oxidative stress as shown by increased lipid peroxidation, free radical generation, and arachidonic acid release with decreased glutathione. Biological compounds with antioxidant properties and renal membrane-regenerating potential may be a boon in alleviating adenine-induced toxicity.

Laminaria japonica Aresch has been utilized in China for over one thousand years. It was documented in Traditional Chinese Medicine that it has therapeutic effect on hydropsy, a symptom of renal failure (Yen, 1996). Fucoidans are highly sulfated cell-wall polysaccharides found mainly in various species of brown seaweeds such as Kombu, Undaria pinnatifida, and Sargassum C. Ag., and variant forms of fucoidan have also been found in animal species, including the sea cucumber (Bilan & Usov, 2008). Recently, substantial pharmaceutical researches have been done on fucoidan. As a consequence of these researches, fucoidan is now being marketed as a nutraceutical and food supplement (Clement et al., 2010; Kim, Lee, & Lee, 2010). Low molecular weight fucoidan (DF) is the fragment of unfractionated fucoidan obtained by either chemical/enzymatic depolymerization. In comparison to native fucoidan, the advantages of DF include a higher degree of bioactivity and easier absorption (Zhu et al., 2010). Accordingly, recent studies have corroborated a renoprotective role for fucoidan in animal models of kidney injury. The supplement of fucoidan to rats with chronic renal failure demonstrated the renoprotective effects of fucoidan. Further, it was also found to be inhibiting the development of proteinuria associated with Heymann nephritis (Zhang, Li, Xu, Niu, & Zhang, 2003; Zhang et al., 2005). Veena, Josephine, Preetha, Varalakshmi, and Sundarapandiyan (2006) reported the fucoidan was able to increase the antioxidant enzymes in ethylene glycol (EG) treated rats, and they also found fucoidan could ameliorate the oxalate-induced peroxidative injury. Thus, antioxidants are expected to decrease the vulnerability of the kidney to oxidative challenges. The excellent antioxidant activity of DF and UF suggested that it may be a useful drug for treating CKD complications.

Abbreviations: CKD, chronic kidney disease; DHA, 2,8-dihydroxyadenine; SCR, serum creatinine; SUN, serum urea nitrogen; CAT, catalase; GSH-PX, glutathione peroxidate; GSH, glutathione; LPO, lipid peroxidation; MDA, malondialdehyde; ROS, reactive oxygen species; BSA, bovine serum albumin; DF, low molecular weight fucoidan; UF, high uronic acid fucoidan; HK, Haikunshenxi capsule, a medicine used on the clinic in China.

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To our knowledge, despite a broader understanding concerning the renoprotective properties of fucoidan, being achieved in the recent years, the anti-free actions of the fucoidan in CKD state is fairly unknown. This work focuses on the antioxidant and renoprotective actions of DF and UF in an adenine-induced CKD model, where both the renal damage and the oxidative stress have a larger role to play. The present study was conducted with an aim to examine whether fucoidan, could ameliorate the adenine-induced CKD by assessing the activity of antioxidant enzymes and decreasing the LOP level along with histopathological studies.

2. Materials and methods

2.1. Materials

L. japonica Aresch (Laminariaceae), cultured at the coast of Qingdao, China, was collected in August 2008, authenticated by Prof. Lanping Ding and stored as a vouvher specimen (No. 80) in the Jerbarium of the Algal Chemistry Department, Institute of Oceanology. The fresh seaweeds were soon washed, sun dried and kept at room temperature for use. Fucoidan and DF were prepared as described previously (Wang, Zhang, Zhang, & Li, 2008; Wang et al., 2009). Briefly, fucoidan was extracted with hot water and purified using 3600 Da Mw cutoff dialysis membranes, then precipitated with ethanol (75%, final concentration) and dried (Wang et al., 2008). DF was prepared using ascorbate and hydrogen peroxide (30 mmol/L, 1:1), after reaction for 2 h, the solution was dialyzed using 3600 Da Mw cutoff dialysis membranes and precipitated with ethanol (Wang et al., 2008; Wang et al., 2009). UF was performed using anion-exchange chromatography according to the previous method (Wang et al., 2008). In brief, DF was dissolved and applied to a column of DEAE-Sepharose CL-6B, and eluted with 0.5 M NaCl solution, the elution was dialyzed, concentrated and finally lyophilized in a freeze dryer (Tokyo Rikakikai Co., Ltd., Japan). The backbone of DF was presumed as following (Wang et al., 2010).

2.2. Animals

Wistar male rats (SXKLu20080002), weighting approximately 180–220 g, were purchased from Laboratory Animal Center, Shandong University of Traditional Chinese Medicine, China. The animals were maintained under standard conditions of humidity, temperature (24 ± 1 °C) and light (12 h light/12 h dark). The animals were fed with standard rat diet containing 20% protein (Laboratory Animal Center, Shandong University of Traditional Chinese Medicine) and water ad libitum during the experiments. In this experiment, all drugs were administered orally to the stomach through an intragastric tube. Animals were maintained according to the National Guide for the Care and Use of Laboratory Animals.

2.3. Experimental procedure

CKD model was induced by adenine (300 mg/kg/d) through an intragastric tube to the stomach for four weeks. At the end of the fourth week, we tested the SUN and SCR in the blood serum; and the results confirmed the CKD model was ready. CKD rats were randomly divided into 6 groups ($n = 12$). One was control group; one was positive control group HK (Haikunshenxi capsule, 150 mg/kg/d), DFL (DF 50 mg/kg/d), DFH (DF 150 mg/kg/d), UFL (UF 50 mg/kg/d) and UFH (UF 150 mg/kg/d) were administered orally to the other four groups for 28 days, respectively. HK (Haikunshenxi capsule) is a medicine used on the clinic in China, state drug approval document No.: Z20030052. The main composition of HK is sulfated polysaccharide extracted from *L. japonica*, the main chemical composition of the HK is as following: fucose: 25–30%, sulfate group: 27–33%, uronic acid: 2–4%, other monosaccharides such as galactose, mannose, rhamnose: 7–12%. The medicine is produced in Huinan Changlong Co. Ltd., Jilin, China. Control and normal rats were treated with the same volume of distilled water. The animals were anaesthetized by chloral hydrate and the blood samples were taken from heart on the 28th experimental day, and the body and kidney weights of rats were monitored. After blood was taken, the rats were killed and the kidneys were taken and fixed in 10% formalin. Livers were homogenized (1:10, w/v), in ice-cold 0.86% NaCl solution. Homogenates were centrifuged at 9000 × *g* for 20 min at 4 °C to remove cell debris and then supernatant were collected and stored at −80 °C until employed later to determine total protein content, enzymatic activities/level (including GSH-PX, CAT, GSH) and MDA.

2.4. Analytical methods

The fucose content was determined according to the method of Gibbon (1955) using fucose as standard. Sulfate content was analyzed by the barium chloride-gelatin method of Kawai, Seno, and Anno (1969). Uronic acid was estimated in a modified carbazole method using D-glucuronic acid as standard (Bitter & Muir, 1962). For the determination of neutral sugar composition, the acid hydrolyzed glycoses were converted into their 1-phenyl-3-methyl-5-pyrazolone derivatives and separated by HPLC chromatography (Honda et al., 1989). The molecular weight of samples were assayed by HP-GPC system (Zhao et al., 2006).

2.5. Assessment of renal function

SCR, SUN, serum total protein and albumin were analyzed with Roche Modular biochemical analyzer. The protein of the liver was determined estimated using Coomassie Brilliant Blue (G-250) by the method of Bradford (Bradford, 1976) using a bovine serum albumin (BSA) standard.

2.6. Assessment of enzymatic activities/levels

The serum and liver activities/levels of enzymes (GSH-PX, CAT and GSH) and MDA were analyzed using kits from NanJing JianCheng (NanJing JianCheng Bio Inst, China), and the protocols were all followed the introduction in kit, except the volume of reagent and samples were scaled down to yield a final reaction system of 200–300 μL, viz., the volume mentioned in kit was divided by a factor of 10 or 20 for each enzyme. All antioxidant enzyme activities were determined spectrophotometrically in a microtiter plate reader (Gene5, Gene Company Limited, China) at constant temperature (20 °C).

2.7. Histopathological studies

A portion of kidney tissue was fixed in 10% formalin. Tissues were washed and dehydrated in descending grades of isopropanol and finally cleared in xylene. The tissues were then embedded in molten paraffin wax. Sections were cut at 5 μm thickness, strained with haematoxylin and eosin. The sections were analyzed under light microscope for histopathological changes.

2.8. Data statistical analysis

The results were expressed as the means ± S.D.; and the statistical significance of differences between means was estimated by ANOVA using SPSS software for windows (ver. 13.0); $P < 0.05$ was considered to be statistically significant.

Table 1
Chemical composition (%, dry weight) and molecular weight of DF and its fraction (UF) isolated from *L. japonica*.

Sample	Fucose	Uronic acid	Sulfate	Molecular weight (Da)	Neutral sugar					
					Fuc	Gal	Man	Glc	Rha	Xyl
DF	28.7	3.65	30.1	6,500	51.5	32.7	2.13	9.01	3.07	1.54
UF	25.6	13.0	21.1	9,544	42.7	31.9	5.71	12.4	4.23	3.17

3. Results

3.1. Chemical analysis

The chemical composition of DF and UF is shown in Table 1. The result showed that the main chemical components of DF and UF were fucose and sulfate, along with uronic acid. The content of fucose and sulfate group in DF was a little higher than those in UF, however, the uronic acid in UF was higher than that in DF significantly. Meanwhile, the neutral monosaccharides constituents of DF and UF were complex. Results showed that fucose and galactose were the main sugar form accounting for 84.24% and 74.53% of total neutral sugar in DF and UF. Additional to fucose and galactose, other monosaccharides such as mannose, glucose, rhamnose and xylose were also seen in the DF and UF. The molecular weight of DF and UF was 6500 Da and 9544 Da, respectively.

3.2. Body weight and the ratio of kidney weight/body weight alterations

The changes in body weight and the ratio of kidney weight/body weight were examined in normal and experimental animals (Fig. 1). Significant changes in body weight and the ratio of kidney weight/body weight were observed in control and other groups during treatment period. Body weight was significantly lower ($P < 0.05$) in the control group, and the ratio of kidney weight/body weight was significantly higher ($P < 0.05$) in the control group compared to the normal and other treatment groups, respectively. However, UFH, DFL and DFH administered groups showed minimal alterations in both the parameters compared to the normal group.

3.3. Assessment of renal function

The SCR and SUN levels of adenine induced CKD rats were significantly higher than those of the normal rats ($P < 0.01$, Fig. 2.). After treatment with DF and UF for 28 days, the SCR and SUN levels of treated rats were evidently lower than those of control rats ($P < 0.01$). DF and UF at the dose of 150 mg/kg had stronger renoprotective effects than those of positive control at the same dose.

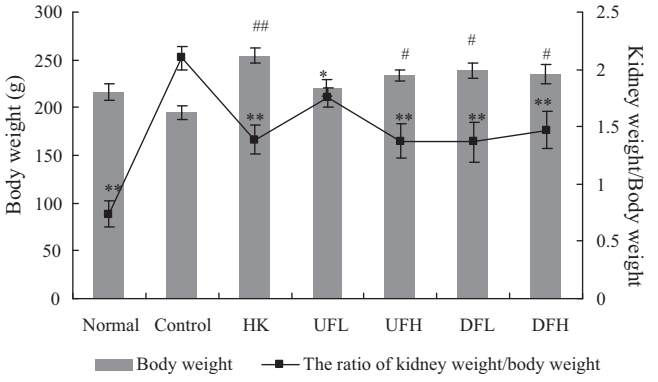

Fig. 1. The body weight and the ratio of kidney weight/body weight in adenine induced CKD rats at the end of the experiment. *$P < 0.05$ and **$P < 0.01$ (vs. control group).

Fig. 3 depicted the histopathological alterations and the effect of DF and UF administration of all the normal and experimental rats. Histopathological sections from normal rats showed normal glomeruli with tubules. The control rats exhibited severe glomerulotubular damage and necrosis with numerous crystal deposited. Also, there was formation of foreign body granuloma in the renal tubules and interstitum, and marked fibrosis leading in some extreme cases to normal kidney. The rats of DF and UF treatment group displayed mild tubular damage with few crystals in the tubular lumen. The rats of DFL treatment group exhibited marked tubular denudation with glomerular congestion.

3.4. Assessment of enzymatic activities/levels

The comparison of the CAT activity in serum and liver in experiment groups is summarized in Fig. 4. The activity of CAT in serum was significantly lower in the control group than in the normal group ($P < 0.01$). UFH treatment could increase the CAT level in serum obviously ($P < 0.01$), however, other treatment groups had little effect on CAT activity; some groups even had lower CAT activity than the control group. CAT level in liver was decreased in the control group when compared with the normal group ($P < 0.01$). The

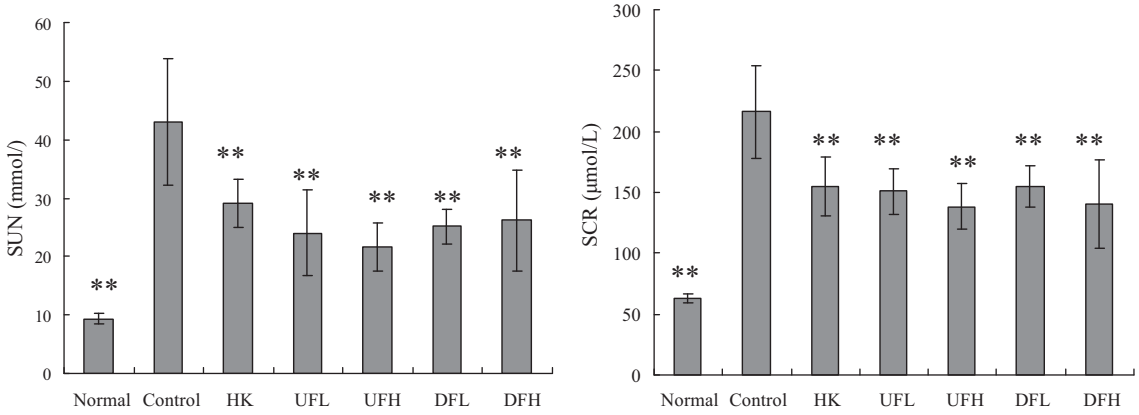

Fig. 2. Effects of the DF and UF on the serum biochemical levels (SUN and SCR) in adenine induced CKD rats at the end of the experiment.

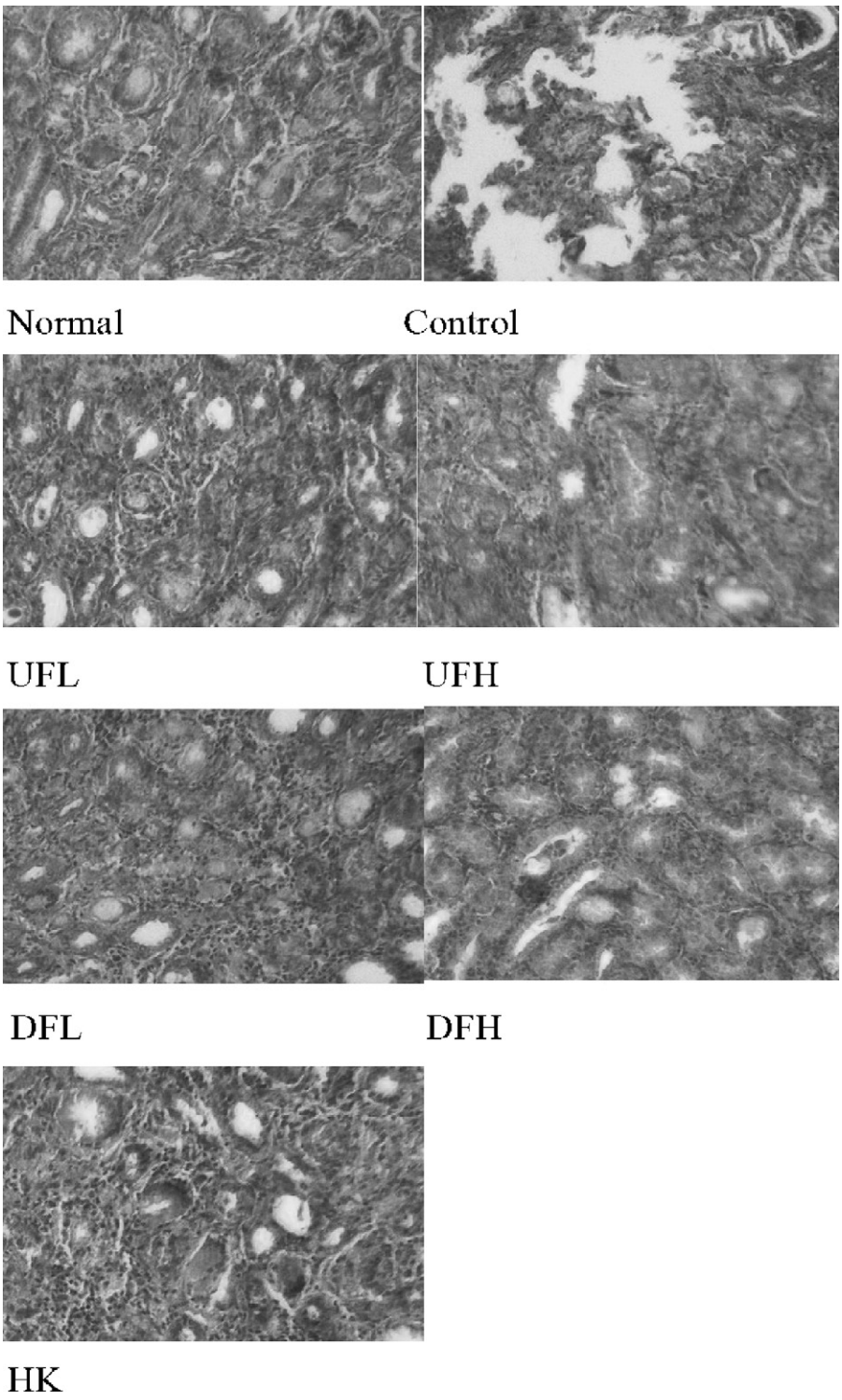

Fig. 3. Histopathological studies on renal tissues of CKD rats (H&E 100×).

CAT level in liver was increased significantly in DF and UF treatment group ($P < 0.01$ and $P < 0.05$, respectively). DFL and DFH treatment group had the similar activity of CAT, which was higher than that of the HK treatment group.

The decreased activities of GSH-PX were observed in serum and liver in the control group when compared to the normal group (Fig. 5.). DF and UF could increase the GSH-PX level in the treatment rats in both serum and liver. The influence was dose-dependent in the treatment groups of GSH-PX in liver, DFH treatment group exhibited most excellent activity among all the groups.

The GSH level in the serum and liver in experiment rats are described in Fig. 6. The GSH level in serum and liver in the control group were lower than in the normal group, respectively. All the treatment groups could increase the GSH level, some of them were significantly ($P < 0.01$ or $P < 0.05$).

Serum MDA levels were increased in the control group when compared with the normal group (Fig. 7). Significant reduction

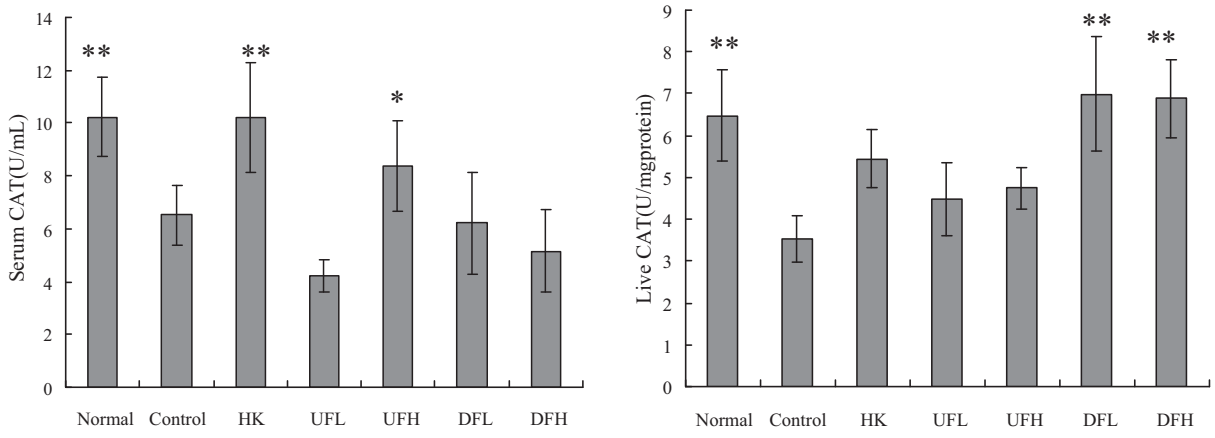

Fig. 4. CAT activity in the blood serum (left) and liver (right) in the normal, control, UFL, UFH, DFL and DFH group rats at the end of the experiment. $^{*}P < 0.05$ and $^{**}P < 0.01$ (vs. control group).

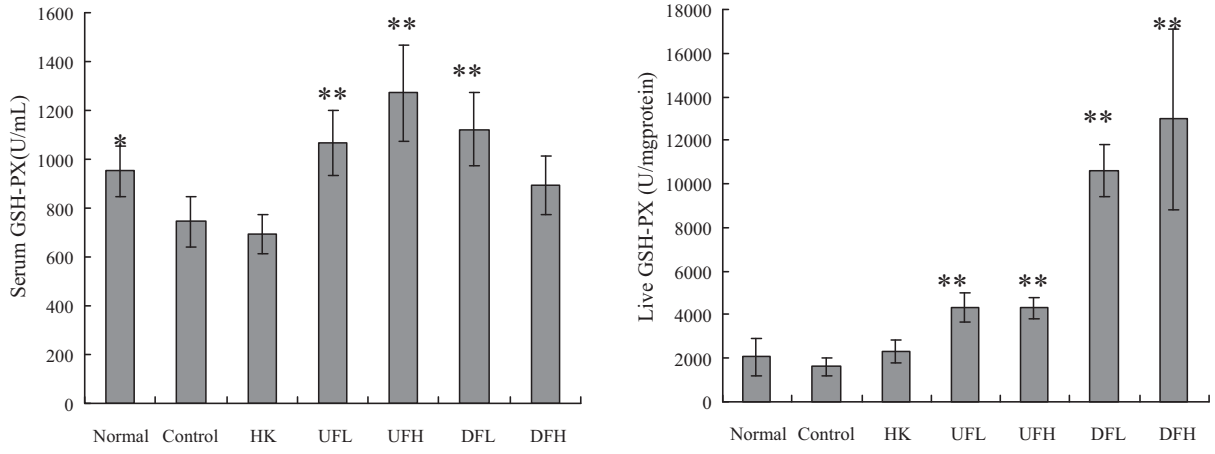

Fig. 5. GSH-PX activity in the blood serum (left) and liver (right) in the normal, control, UFL, UFH, DFL and DFH group rats at the end of the experiment. $^{*}P < 0.05$ and $^{**}P < 0.01$ (vs. control group).

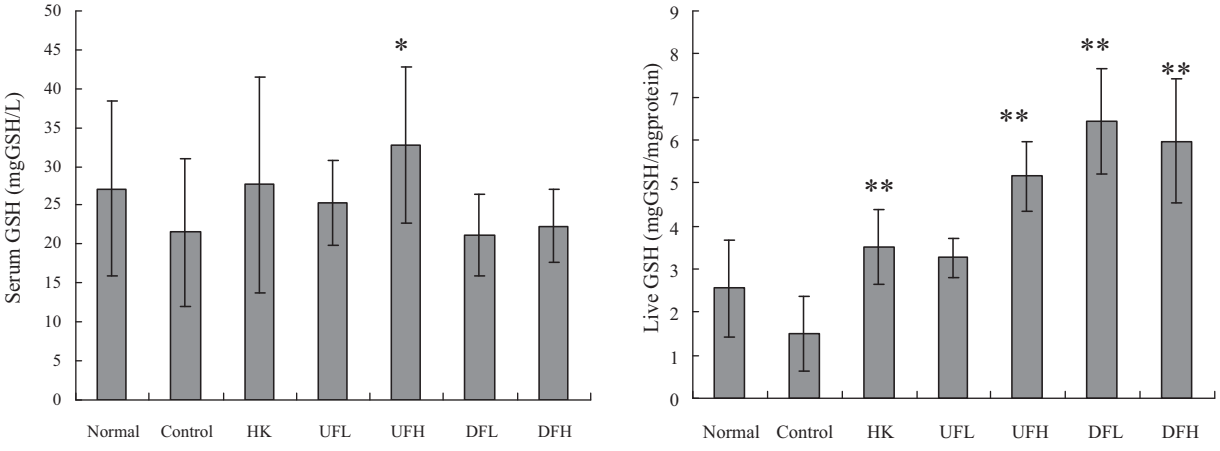

Fig. 6. GSH activity in the blood serum (left) and liver (right) in the normal, control, UFL, UFH, DFL and DFH group rats at the end of the experiment. $^{*}P < 0.05$ and $^{**}P < 0.01$ (vs. control group).

in the MDA levels were observed in DF and UF treatment group, respectively ($P < 0.05$ and $P < 0.01$, respectively). Liver MDA in control group was significantly higher than in the normal group ($P < 0.01$). All the drug treatment groups could diminish the content of MDA, especially the HK and UFL group.

4. Discussion

We examined the renal function of the rats including SCR and SUN. SCR demonstrates the ability of kidney to remove creatinine from the blood and concentrate it in the urine. Renal dysfunc-

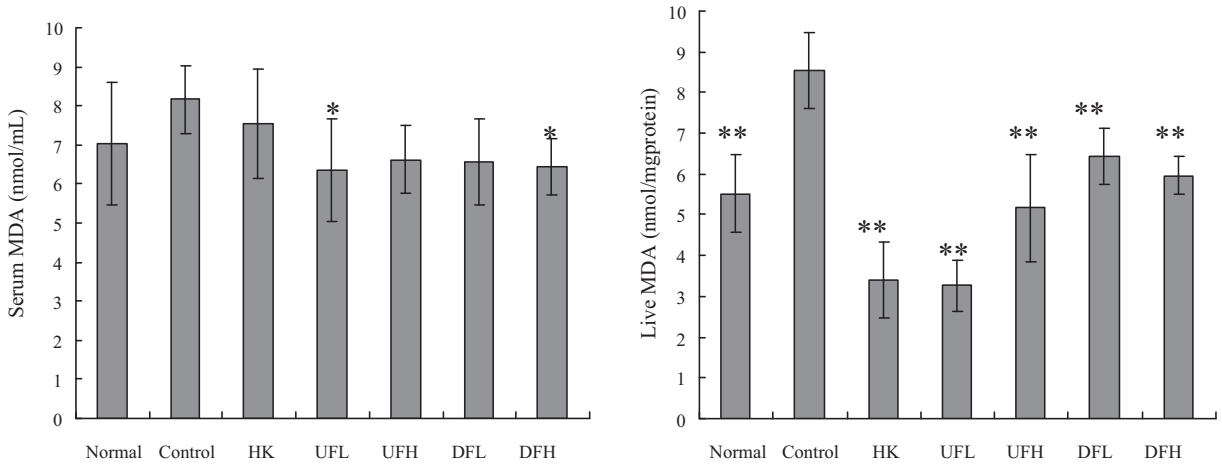

Fig. 7. MDA concentration in the blood serum (left) and liver (right) in the normal, control, UFL, UFH, DFL and DFH group rats at the end of the experiment. $^{*}P<0.05$ and $^{**}P<0.01$ (vs. control group).

tion diminishes the ability to filter creatinine and so the SCR rises. Diseased or damaged kidneys cause an elevated SUN because the kidneys are less able to clear urea from the bloodstream (He et al., 2009). A fall in the degree of SUN and SCR in rats receiving DF and UF further substantiates its beneficial effect. Many experimental studies have demonstrated that fucoidan decrease proteinuria and slow down the progression of diabetic nephropathy possibly by their haemodynamic actions and anionic properties. It was also confirmed that sulfated polysaccharide protect the remnant kidney by mechanisms independent of glomerular haemodynamic changes, probably thanks to its inhibition of mesangial cell proliferation. Modulation of synthesis and composition of the ECM may play a role as well (Ceol et al., 2000). It was claimed that these beneficial effects of sulfated polysaccharide were mediated by replacing the electronegative content of the glomerular cells (Gambaro et al., 1999).

Histopathology observation of CKD renal sections showed dilated collecting systems with deposition of crystals, which means the adenine was oxidized to DHA, and the glomeruli and tubules was damaged at some extent. The DHA crystals could enhance the production of peroxides and toxic superoxide anion radicals (Veena et al., 2006). Free radicals interact with renal epithelia damaging the renal membranes and lead to the tubules dysfunction or damage (Grases, Garcia-Ferragut, & Costa-Bauza, 1998). DF and UF could scavenge the free radical and enhance the activity of the antioxidant enzymes, so the histopathology of treatment renal sections alleviated the tubules dysfunction or damage.

Oxidative stress results from the excessive generation of oxidants, which overwhelms antioxidant defense mechanisms. Altered antioxidant enzymatic and non-enzymatic system function was observed in the CKD rats. The negative correlation was found in our study in plasma SUN, SCR and antioxidant enzymes in rats treated with adenine demonstrated that free radical production activation is highly influenced by the damage of renal. A possible mechanism of DF and UF on CKD is by its effect on the antioxidant enzyme and non-antioxidant enzyme activity (Veena, Josephine, Preetha, & Varalakshmi, 2007). The liver is one of the major oxidant defense organs, so it has numerous enzymatic antioxidants and non-enzymatic antioxidants, so we tested the activities/levels of enzymatic antioxidants and non-enzymatic antioxidants in the experimental rats. The current study illustrated the antioxidant properties of DF and UF could reduce oxidative stress in the serum and liver as seen in increasing antioxidant enzyme activity and decreasing MDA concentration during DF and UF treatment.

CAT is an inducible cytosolic enzyme which has functions to protect the biological system against ROS (Roméo, Bennani, Gnassia-Barelli, Lafaurie, & Girard, 2000). CAT can facilitate the removal of hydrogen peroxide (H_2O_2), which is metabolized to oxygen (O_2) and water (H_2O). Decrease in the activity of CAT might be attributed to direct inhibition of CAT by oxalate and decreased regeneration of CAT from its inactive form, due to lesser availability of NADH (Kirkman & Gaetani, 1984). DFL and DFH could enhance the activity of CAT greatly in liver, so they could mitigate the peroxidative in CKD rats. GSH-PX could break down H_2O_2 and other peroxide, notably those derived from the oxidation membrane phospholipids, into water and oxygen, thus limiting the production of the highly reactive hydroxyl free radical. GSH-PX acting in tandem provides the primary enzymatic antioxidant defenses (Amstad, Moret, & Cerutti, 1994; CeballosPicot et al., 1996). All the samples could increase the activity of GSH-PX, especially in the liver. The activities of GSH-PX in the liver of the DFL and DFH treatment groups were nearly six–seven folds higher than in the normal and control group, which was to say, DFL and DFH could inhibit the generation of the ROS from H_2O_2 and reduced the lipid peroxide.

Reduced glutathione (GSH), an important oxidant defense, functions in the reduction of oxidized tissue components. The observed decreasing of GSH in control rats might be due to its increased conversion to GSSG. Hogberg reported that GSH depletion induces LPO and ultimately cell lyses. Replenishing the GSH levels is, therefore, necessary for the maintenance of the overall thiol status in the cell (Hogberg, Larson, Kristofe, & Orrenius, 1974). Indeed, GSH concentration closely correlated with the degree of renal failure. Depletion in GSH by itself could contribute to the progression of uremia because it has been demonstrated that GSH depletion in rats leads to an acute renal failure (Abulezz, Walker, & Shah, 1991). Moreover, GSH also acts as a radical scavenger by itself and as a detoxicant in eliminating different electrophilic compounds of exogenous and exogenous toxic compounds (CeballosPicot et al., 1996). All the tested samples could increase the activity of the GSH both in the serum and in the liver. The effect in the liver was more obviously than in the serum, DFL and DFH had better effect than UFL and UFH, respectively.

The formation of MDA, a thiobarbituric acid reactive end product served as the index of LPO. Many authors have found a reduction in the levels of different antioxidant enzymes of patient with CKD (Ozden, Maral, Akaydin, Cetinalp, & Kalender, 2002; Richard et al., 1991; Zwołińska, Grzeszczak, Szczepańska, Kiliś-Pstrusińska, & Szprynger, 2006). In our study, we found DF and UF could increase the activity of such as CAT and GSH-PX, and lower the level of the

MDA especially in the liver. The effect of all the samples was similar and dose-independent. The effect on the antioxidant enzymes in the liver was greater than in the serum, therefore, the effect on the MDA in the liver was better than in the serum.

5. Conclusion

The above changes allow us to be certain that adenine-induced peroxidative processes are notably lead to renal tissue damage in our experimental model. This also ascertains the antioxidant potential of DF and UF for the first time in record in countering the adenine-induced oxidative challenge in experimental CKD. This study proved the mechanisms underlying the protective action of DF and UF were related to their antioxidant activity.

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