Effects of Decoction and Powder of Sipjotang with Jujubae fructus or Licorice on Liver and Kidney

In Gyo Seo[1], Rong Jie Zhao[1,2], Eun Young Park[1,3], Sang Mi Park[1,3], Song I Choi[1], Han Kyun Kim[1], Sook Jahr Park[1,3], Young-Kyu Kwon[1,3], Sang Chan Kim[1,3*]

1:College of Oriental Medicine, Daegu Haany University, 2:College of Medicine, Yanbian University, Jilin, China.
3:Development Team for The New Drug of Oriental Medicine (BK21 Program), Daegu, Korea

The effects of Jujubae Fructus and Licorice extracts on the main components of Sijotang Euphorbiae Kansui Radix, Daphinis Genkwa Flos, Euphonrbiae Pekinensis Radix (KWD) treatment [KWD decoction (KWDD) and KWD powder (KWDP)] related toxicities were examined in the kidney and the liver. To select more suitable extract which effectively reduce KWD-treatment related toxicities in the body, blood biochemical and histopathological changes induced by KWD were analyzed in the rats which received treament of KWD + Jujubae Fructus or KWD + Licorice. In the present study, no KWD-treatment related blood biochemical and histopathological change in the liver was detected. However, increase of tubules containing hyaline casts and atrophic tubules in the kidney was detected as the indicators of KWDD treatment related nephrotoxicity. Addition of Jujubae Fructus (KWDDJ) or Licorice (KWDDL) extracts effectively inhibited the nephrotoxcity induced by KWDD treatments. More ameliorated effects were acquired by addition of Jujubae Fructus extract (KWDDJ) than Licorice (KWDDL). In KWDP treatment, there was no significant difference in the number of tubules containing hyaline casts in all drug treated groups compared to normal or control group except for high dose of KWDP. Both of Jujubae Fructurs and Licorice reduced high dose of KWDP treatment related nephrotoxicity, and there was no significant difference between KWDPJs and KWDPLs. It is concluded that addition of Jujubae Fructus is more suitable than Licorice in reducing the nephrotoxicty of KWDD, also it is more suitable to taking Sipjotang in the form of powder than decoction.

Key words : Sipjotang, Licorice, Jujubae Fructus, Toxicity, Kidney, Liver

Introduction

Sipjotang, one of the formulas collected and recorded in Chinese medical classic "Sanghanron", is widely used to treat edema via drastically expel the excessive fluid[1].

This formula, Sipjotang, is used for pleural effusion and edema, manifested by pain in the chest and hypochondrium when coughing or spitting, hardness and stuffiness in the epigastrium, retching, shortness of breath, headache with halo, difficult respiration due to pain in the thorax and back, slippery coating on the tongue, sunken and taut pulse; or edema which is more serious in the lower part of the body, abdominal fullness, dyspnea, difficulty in urination and defecation[2,3].

The modified formula is also used to treat hydrothorax, ascites and edema caused by exudative pleurisy, hepatic cirrhosis, acute pancreatitis, chronic nephritis and nephrotic syndrome, etc.; or simple intestinal obstruction, acute gastric dilatation and others that are ascribed to strong body resistance with exuberance of pathogenic factors[4-6].

Sipjotang are usually composed of four herbs such as Euphorbiae Kansui Radix, Daphinis Genkwa Flos, Euphonrbiae Pekinensis Radix (in identical ratio) and ten grains of Jujubae Fructus[2] (the name of Sipjotang comes form it)[7]. Since three of Euphorbiae Kansui Radix, Daphinis Genkwa Flos, Euphonrbiae Pekinensis Radix (these three herbs are abbreviated into KWD in the following article) are poisonous and act in drastic way, it needs to add ten grains of Jujubae Fructus to buffer the toxicities and drastic action[3,6].

Generally, licorice is widely used to moderate other herbs' toxicities[8] in Korean and Chinese traditional medicine, but in this formula Jujubae Fructus is added to act as the toxicities' buffer.[1-3,6]

Traditionally there are two empirical consensuses: 1. the

* To whom correspondence should be addressed at : Sang Chan Kim, College of Oriental Medicine, Daegu Hanny University, 165 Sang-dong, Suseong-gu, Daegu 706-828, Korea
· E-mail : sckim@dhu.ac.kr, · Tel : 053-770-2247
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toxicities of Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix are increased when combined with licorice. 2. Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix must be administered in the form of powder other than in the form of decoction.

To evaluate the two empirical consensuses, in this study 1. we compared the effects of three crude extracts from Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix boiled in pure water, boiled in the medical solution obtained from boiling Jujuae fructus in pure water (details see methods) and boiled in the medical solution obtained from boiling licorice in pure water (details see methods) on rats' livers and kidneys. 2. We also compared the toxicities of powder form when administered together with water, medical solution from Jujubae Fructus or licorice on rats' livers and kidneys respectively.

Materials and Methods

1. Experimental Parts

1) Part I : Administration of KWD Decoctions

Three different Sipjotang decotions were obtained by boiling Euphorbiae Kansui Radix (Korean name; Kamsu), Daphinis Genkwa Flos (Korean name; Wonhwa), Euphonrbiae Pekinensis Radix (Korean name; Daegeuk) in pure water, medical solution of Jujuae fructus (MSJ) and medical solution of Licorice (MSL) respectively. (Table 1)

2) Part II: Administration of KWD Powder

KWD powder was administered together with water, MSJ or MSL. (Table 1)

Table 1. Experimental groups in the study.

Type of formula	Group	drugs	Dose*
Part I decoction	Normal	-	-
	Control	water	5ml/kg
	KWDD1	KWD decoction (boiled in water)	0.067g/5ml/kg
	KWDD2	KWD decoction (boiled in water)	0.67g/5ml/kg
	KWDDJ1	KWD decoction (boiled in MSJ)	0.067g/5ml/kg
	KWDDJ2	KWD decoction (boiled in MSJ)	0.67g/5ml/kg
	KWDDL1	KWD decoction (boiled in MSL)	0.067g/5ml/kg
	KWDDL2	KWD decoction (boiled in MSL)	0.67g/5ml/kg
Part II powder	Normal	-	-
	Control	water	5ml/kg
	KWDP1	KWD powder suspended in water	0.067g/5ml/kg
	KWDP2	KWD powder suspended in water	0.67g/5ml/kg
	KWDPJ1	KWD powder suspended in MSJ	0.067g/5ml/kg
	KWDPJ2	KWD powder suspended in MSJ	0.67g/5ml/kg
	KWDPL1	KWD powder suspended in MSL	0.067g/5ml/kg
	KWDPL2	KWD powder suspended in MSL	0.67g/5ml/kg

KWDD: Decoction of Kamsu, Wonhwa and Daegeuk. KWDDJ: KWDD + Jujubae Fructus. KWDDL: KWDD + Licorice. KWDP: KWD Powder. KWDPJ: KWDP + MSJ. KWDPL: KWDP + MSL. *: weight of dried herb.

2. Preparation of Decoction and Powder

1) Preparation of KWD decoction (KWDD)

The composition of Sipjotang originates from Sanghanron, a Chinese medical formular classic. KWD decoctions were prepared by boiling KWD in water, MSJ or MSL. For example, 300g of KWD was boiled in 2L of water, and condensed into final concentration of 1 g/ 5ml. MSJ and MSL were prepared by boiling 1500g of Jujubae Fructus or Licorice in pure water respectively, the concentration of them are 0.75 g / ml. All herbs used in this study were purchased from Daewon pharmacy (Daegu, Korea).

2) Preparation of KWD powder (KWDP)

KWD powder (KWDP) was prepared by grinding KWD with a commercial electrical pulverizer.

3. Animals

Animal studies were conducted in accordance with the institutional guidelines for care and use of laboratory animals. Sprague - Dawley male rats weighting 140 - 160 g (Samtako Co., Osan, Korea) were housed under the supply of filtered pathogen-free air, given access to commercial rat chow (Purina, Korea) and water ad libitum. The room was maintained at temperature between 20 and 23°C with 12 h light - dark cycle, relative humidity was 50%. Before being manipulated all rats were acclimatized for 1 week.

4. Administration

1) Part I : In decoction treatment, all drugs were orally given to the rats once a day (doses see table 1) for 3 consecutive days.

2) Part II : In powder treatment, KWD powder was suspended in water, MSJ and MSL, and were orally administered to rats once a day for 3 days.

5. Blood Biochemical Analysis

24 h after being given the last dose of respective drugs rats were sacrificed. The blood samples were collected from rats' heart (details were omitted) and assigned to the analysis for Alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), and lactate dehydrogenase (LDH) using Spectrum, an automatic blood chemistry analyzer (Abbott Laboratories, Abbott Park, IL).

6. Histological Analysis

24 h after being given the last dose of respective drugs rats were sacrificed. Rats' liver and kidney parenchyma were separated, fixed in 10% neutral buffered formalin (NBF), embedded in paraffin, sectioned (3 ~ 4μm) and stained with Haematoxylin & Eosin (H&E) stain.

7. Histomorphometrical Analysis

Numbers of tubules having hyaline casts with or without atrophic changes were calculated using automated image analysis (analySIS Image Processing; SIS, Germany) as N/1000 total observed tubules.

8. Statistical Analysis

All numerical data were shown as Mean ± S.D. (n=9). Statistical analysis was conducted using Mann-Whitney U-Wilcoxon Rank Sum W test (MW test) with SPSS for Windows (Release 6.1.3., SPSS Inc., USA). Additionally, % changes between KWD (i.e. KWDD or KWDP) groups in different doses or control group, and between KWD groups, and other groups were calculated by the following way.

- % Changes between vehicle control and KWD groups = {(Data of KWDD groups - Data of Control)/Data of control} × 100
- % Changes between KWD groups and test groups (%) = {(Data of KWD groups - Data of test groups)/Data of KWD groups} × 100

Results

1. Part I : Effects of Sipjotangs on Liver and Kidney of Rats When Administrated in the Form of Decoction.

1) Blood Biochemical Analysis

In clinical examination, the increased levels of AST, ALT and LDH in plasma are considered as biomarkers of hepatic injury, and the high level of BUN usually indicates abnormal function of kidney. So analysis of ALT, AST, LDH and BUN was conducted to evaluate the possible injury to livers and kidneys caused by taking Sipjotang.

As seen in Table 2, there was no significant change in the level of ALT, AST, LDH and BUN in all groups of KWDDs, KWDJs and KWDLs. Statistical analysis showed $p > 0.05$.

Table 2. Blood biochemical analysis in the rats administrated in the form of decoction of Sipjotang.

	AST	ALT	LDH	BUN
NOR	97.50±23.64	54.16±15.52	518.11±109.12	19.75±2.25
CON	105.95±31.49	52.43±21.49	568.45±156.44	19.00±2.93
KWDD1	109.43±24.98	50.43±16.00	503.68±165.37	21.88±2.42
KWDD2	82.55±24.84	61.78±18.07	369.78±70.63	20.00±2.83
KWDDJ1	116.72±40.07	64.66±19.26	484.86±102.47	16.50±3.07
KWDDJ2	85.85±32.59	61.03±10.99	399.05±139.09	16.50±2.00
KWDDL1	109.63±52.27	62.74±18.15	551.68±151.58	20.00±2.78
KWDDL2	82.05±33.83	39.01±14.38	497.99±147.75	17.50±2.62

Blood samples were obtained 24 h after the final treatment of Sipjotang decoction. Values represent mean ± S.D., 9 rats in each group.

2) Histological Analysis

(1) Histopathological Analysis

① Liver : In groups of KWDDs, KWDDJs and KWDDLs, there were no histopathological changes detected compared to normal or control group in the doses used in the present study. All animals showed relatively normal histopathological features, statistical analysis showed $p > 0.05$ (Fig. 1a-h).

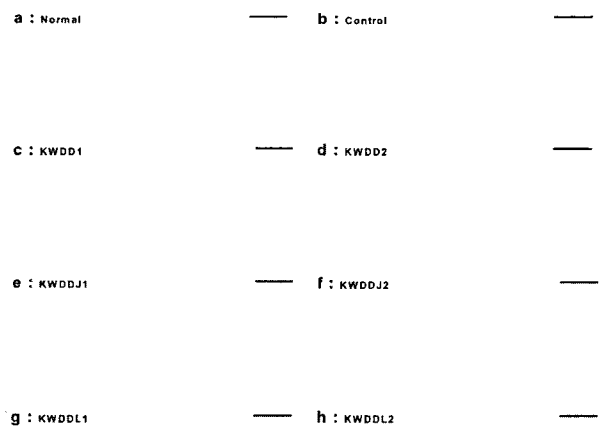

Fig. 1. Histological profiles of the liver in normal (a), control (b), KWDD1 (c), KWDD2 (d), KWDDJ1 (e), KWDDJ2 (f), KWDDL1 (g) and KWDDL2 (h) groups. No histopathological changes were detected in KWDDs, KWDDJs, and KWDDLs groups compared with those of normal and/or control group regardless of the doses used in the present study (All H&E stain; Scale bars = 100 μm).

② Kidney : More numerous tubules containing hyaline casts were detected in all rats who took Sipjotang with/without focal tubular atrophies compared to those of normal or control group (Fig. 2a and b).

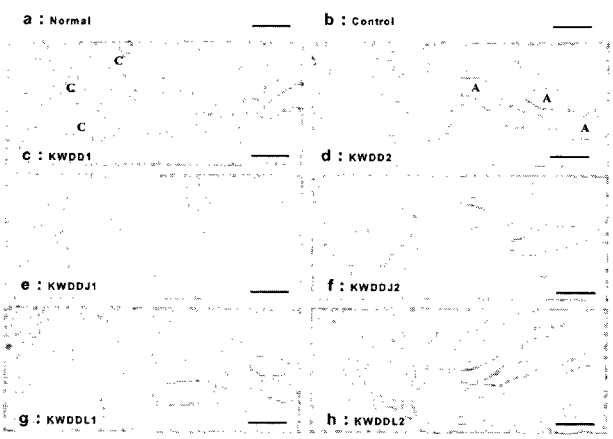

Fig. 2. Histological profiles of the kidney in normal (a), control (b), KWDD1 (c), KWDD2 (d), KWDDJ1 (e), KWDDJ2 (f), KWDDL1 (g) and KWDDL2 (h) groups. Note that there were tubules containing hyaline casts (indicated as C in figure) detected in all groups in this study, some of them with focal tubular atrophies (indicated as A in figure). As seen in Fig. 2, the KWDD-treatment related nephrotoxicities were evident compared to those of normal and/or controls. However, these histopathological changes were dramatically and dose dependently decreased in KWDDJs and KWDDLs groups compared with those of KWDDs groups. More dramatical improvements were detected in KWDDJs than those of KWDDLs groups (All H&E stain; Scale bars = 100 μm).

These histopathological changes in the kidney were dramatically and dose-dependently decreased in KWDDJs (Fig. 2e and f) and KWDDLs (Fig. 2g and h) compared with those of KWDDs groups (Fig 2c and d). Comparing KWDDJs with KWDDLs groups, there were more dramatical improvements detected in KWDDJs groups in both of doses.

(2) Histomorphometrical Analysis -Kidney

① Changes in the numbers of tubules containing hyaline casts (N/1000 tubules)

Compared with control group, the number of tubules containing hyaline casts in KWDDs groups were significantly ($p<0.01$) increased in a dose dependent manner. However, they were significantly ($p<0.01$) decreased in KWDDJs and KWDDLs' groups compared with those of the same dose of KWDDs groups, respectively. Comparing KWDDJs with KWDDLs groups, there were more dramatical decreases of the numbers of tubules having hyaline casts detected in KWDDJs groups in both of doses (Table 3).

Table 3. The histomorphometry analysis in kidney of rats of KWDDs, KWDDJs and KWDDLs

Group	Histomorphometrical Analyses	
	Number of tubules containing casts	Numbers of atrophic tubules
Normal	22.89 ± 6.97	59.22 ± 34.27
Control	23.00 ± 7.25	57.78 ± 41.27
KWDD1	261.67 ± 27.05*	284.22 ± 60.43*
KWDD2	549.33 ± 125.54*	392.56 ± 98.95*
KWDDJ1	133.22 ± 23.46*,#,$$	169.78 ± 31.68*,#,$
KWDDJ2	238.33 ± 92.04*,#,$$$	219.89 ± 55.73*,#,$
KWDDL1	193.33 ± 39.26*,#	198.67 ± 36.25*,#
KWDDL2	391.22 ± 62.02*,#	270.33 ± 76.31*,##

n=9, Mean ± S.D.; * $p<0.01$ compared with that of control by MW test; # $p<0.01$ or ## $p<0.05$ compared with that of the dose of KWDDs (KWDD1 or KWDD2) by MW test. $$ $p < 0.01$ in comparing KWDDL1 with KWDDJ1; $$$ $p < 0.001$ in comparing KWDDL2 with KWDDJ2.

The numbers of tubules having hyaline casts in normal, control, KWDD1, KWDD2, KWDDJ1, KWDDJ2, KWDDL1 and KWDDL2 groups were 22.89 ± 6.97, 23.00 ± 7.25, 261.67 ± 37.05, 549.33 ± 125.54, 133.22 ± 23.46, 283.33 ± 92.04, 193.33 ± 39.26 and 391.22 ± 62.03 tubules/total 1000 observed tubules, respectively. MW test showed that there were significant differences between compared groups. (details see Table 3) The increment of tubules containing hyaline casts in KWDD1 and KWDD2 were 1037.68% and 2288.41% compared to that of control group, respectively. However, the numbers of tubules containing hyaline casts was decreased by -56.61% and -28.78% in KWDDJ2 and KWDDL2 group compared with that of KWDD2 group. In low dose groups, the inhibitory rate was -49.09% and -26.11% in KWDDJ1 and KWDDL1 group compared to that of KWDD1 group.

② Changes in the numbers of tubules showing atrophic changes (N/1000 tubules)

Atrophic tubules in KWDDs groups were apparent ($p<0.01$) and dose dependently increased compared to that of control group. However, they significantly ($p<0.01$) decreased in KWDDJs and KWDDLs groups compared with those of the same dose of KWDDs groups. Comparing KWDDJs with KWDDLs groups, there were more dramatic decreases of the numbers of atrophic tubules detected in KWDDJs groups (Table 3).

The numbers of atrophic tubules in normal, control, KWDD1, KWDD2, KWDDJ1, KWDDJ2, KWDDL1 and KWDDL2 groups were 59.22 ± 34.27, 57.78 ± 41.27, 284.22 ± 60.43, 392.56 ± 98.59, 169.78 ± 31.68, 219.89 ± 55.73, 198.67 ± 36.25 and 270.33 ± 76.31 tubules/total 1000 observed tubules, respectively. The increment of atropic tubules in KWDD1 and KWDD2 was 391.92% and 579.42% compared to that of control group, respectively. However, the numbers of atrophic tubules were decreased by -43.99% and -31.14% in KWDDJ2 and KWDDL2 groups compared with that of KWDD2 group, respectively. In low dose, the inhibitory rates were -40.27% and -30.10% in KWDDJ1 and KWDDL1 group compared with that of KWDD1 group, respectively.

2. Effects of Sipjotang on Liver and Kidney of Rats Administrated in the Form of Powder.

1) Blood Biochemical Analysis

As the same reason in Part I, ALT, AST, LDH and BUN analysis was conducted.

As seen in Table 4, there was no significant change in the level of ALT, AST, LDH and BUN in all groups of KWDPs, KWDPJs and KWDPLs. Statistical analysis showed $p > 0.05$.

Table 4. Blood biochemical analysis in the rats administrated with powder of Sipjotang.

	AST	ALT	LDH	BUN
NOR	79.37±32.58	51.65±9.69	341.47±198.55	14.60±2.30
CON	84.40±21.01	50.45±6.43	401.73±124.90	13.00±3.39
KWDP1	50.45±28.76	42.68±16.25	329.38±149.11	13.17±2.56
KWDP2	56.98±21.59	40.21±13.56	337.15±89.32	12.67±2.88
KWDPJ1	80.26±42.22	42.19±16.81	337.45±99.38	13.67±1.97
KWDPJ2	61.19±19.43	43.38±9.25	334.11±116.76	12.50±3.39
KWDPL1	55.21±23.41	43.31±6.25	344.22±167.89	13.40±2.79
KWDPL2	63.01±26.16	47.10±7.67	339.49±97.13	11.83±3.97

Blood samples were obtained 24 h after final Sipjotang treatment. Values represent mean ± S.D. from 9 rats in each group.

2) Histological Analysis

(1) Histopathological analysis

① Liver : In all powdered Sipjotang groups (KWDPs, KWDPJs and KWDPLs), there were no histopathological changes in the liver detected compared to those of normal or control group. All animals showed relatively normal histopathological features in different doses of Sipjotang powder, statistical analysis showed $p > 0.05$ (Fig. 3a-h).

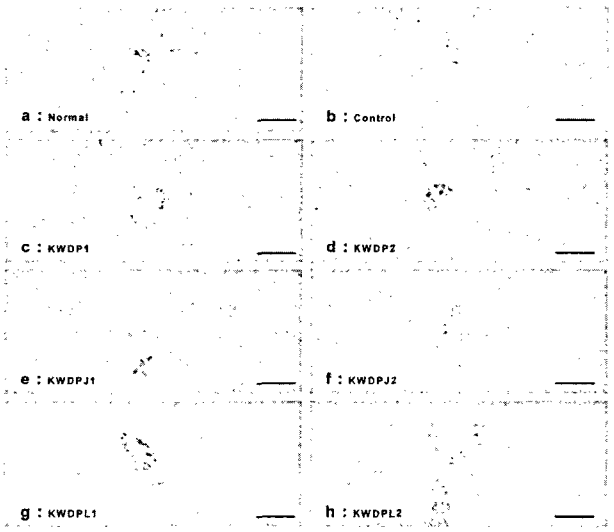

Fig. 3. Histological profiles of the liver in normal (a), control (b), KWDP1 (c), KWDP2 (d), KWDPJ1 (e), KWDPJ2 (f), KWDPL1 (g) and KWDPL2 (h) groups. Note that no KWDP, KWDPJ and KWDPL related histopathological changes were detected compared to those of normal or control group regardless of the doses used in the present study (All H&E stain: Scale bars = 100 μm).

② Kidney : There were no significant histopathological changes in the kidney detected in the groups of KWDP1, KWDPLs and KWDPJs except for KWDP2 group. As seen in Fig. 4. there were numerous tubules containing hyaline casts detected in KWDP2 group, compared to those of normal, control and other groups (Fig 4a-h).

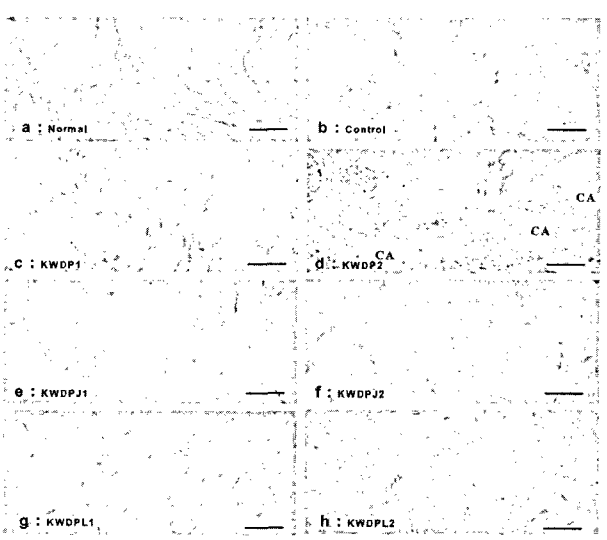

Fig. 4. Histological profiles of the kidney in normal (a), control (b), KWDP1 (c), KWDP2 (d), KWDPJ1 (e), KWDPJ2 (f), KWDPL1 (g) and KWDPL2 (h) groups. Note that increase of tubules containing hyaline casts (indicated as CA in figure) were detected in KWDP2 as KWDP treatment related nephrotoxicities compared to those of normal or control group. However, these histopathological changes were dramatically decreased in KWDPJ2 and KWDPL2 groups compared with that of KWDP2 group. No significant changes were detected in all lower dose groups (KWDP1, KWDPJ1, KWDPL1) (All H&E stain: Scale bars = 100 μm).

(2) Histomorphometrical Analysis -Kidney

① Changes in the numbers of tubules having hyaline casts (N/1000 tubules)

The number of tubules containing hyaline casts in KWDP2 group was significantly ($p<0.01$) increased compared with that of control group. However, there were no significant histolomophometrical change detected in groups of KWDP1, KWDPLs and KWDPJs groups compared with that of control group (Table 5). The numbers of tubules having hyaline casts in normal, control, KWDP1, KWDP2, KWDPJ1, KWDPJ2, KWDPL1 and KWDPL2 groups were detected as 23.80 ± 4.15, 17.60 ± 8.41, 21.17 ± 4.71, 266.17 ± 66.80, 17.17 ± 4.83, 19.00 ± 3.90, 18.83 ± 6.43 and 16.33 ± 3.98 tubules/total 1000 observed tubules, respectively. The increment of the number of tubules containing hyaline casts in KWDP2 was 1412.31% compared to that of control group. However the number of tubules containing hyaline casts in KWDPJ2 or KWDPL2 group was significantly decreased to almost the same level in control group.

Table 5. The histomorphometry analysis in kidney of rats of KWDPs, KWDPJs and KWDPLs

Group	Histomorphometrical Analyses
	Number of tubules containing casts
Normal	23.80 ± 4.15
Control	17.60 ± 8.41
KWDP1	21.17 ± 4.71
KWDP2	266.17 ± 66.80*
KWDPJ1	17.17 ± 4.83
KWDPJ2	19.00 ± 3.90#
KWDPL1	18.83 ± 6.43
KWDPL2	16.33 ± 3.98#

n=9, Mean ± S.D.; * p<0.01 compared to that of control by MW test; # p<0.01 compared to that of KWDP2 by MW test.

3. Comparison of effect of decoction form with powder form of Sipjotang on histopathological change in kidney.

To evaluate whether the form of power is more favorable than form of decoction in term of their effect on kidney, we compared the numbers of tubules containing hyaline casts caused by taking decoction of Sipjotang with powder of Sipjotang.

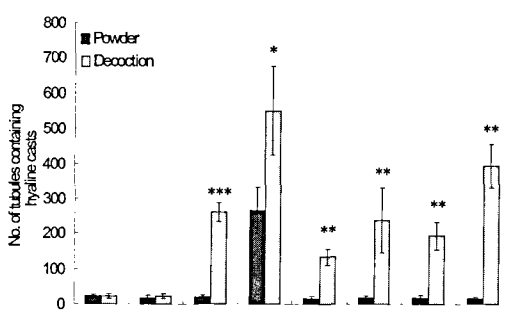

Fig. 5. Comparison of effect of decoction form with powder form of Sipjotang on histopathological change in kidney. Black bars denote numbers of tubules / 1000 tubules containing hyaline casts in powder groups, gray bars denote numbers in decoction groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the respective powder group.

MW test showed that normal group (in decoction part) Vs normal group (in powder part), $p > 0.05$; control group (in

decoction part) Vs normal group (in powder part), $p > 0.05$; KWDD1 Vs KWDP1, $p < 0.001$; KWDD2 Vs KWDP2, $p < 0.05$; KWDDJ1 Vs KWDPJ1, $p < 0.01$; KWDDJ2 Vs KWDPJ2, $p < 0.01$; KWDDL1 Vs KWDPL1, $p < 0.01$; KWDDL2 Vs KWDPL2, $p < 0.01$. (Fig. 5)

Discussion

Sipjotang is originated from Sanghanron written by Zhang Zhong Jing. The formula is well known as a useful formula for treatment of edema via drastically expel the excessive fluid[2,4].

Ryu et al.[9] studied the effect of water extract of Sipjotang on the renal function. They reported that administration of 0.66g/2ml/kg water extract of Sipjotang increased the volume of urine while 1.32g/4ml/kg decreased it. In another study which investigated the effect of Sipjotang on rats' renal function, Kim et al.[10] reported that water extract of Sipjotang ga Buja in doses of 0.79g/2ml/kg and 1.58g/4ml/kg increased the volume of rats' urine.

Other studies done with an individual herb of Sipjotang, Lee et al.[11] investigated the effects of Euphorbia Pekinensis RUPR. on rats with acute renal failure induced by Gentamicin Sulfate; Han et al.[12] investigated effects of Euphorbia Kansui T.N. Liou Ex T.P Wang on rats with acute renal failure induced by Gentamicin Sulfate. In both of the studies they got conclusion that Euphonrbiae Pekinensis Radix and Euphorbiae Kansui Radix without refining have a partial diuretic effect on acute renal failure rats induced by gentamicin sulfate, but aggravated the glomerular filtration rate and tubular resorption.

Additioanlly, Lim et al.[13] studied the influences of Euphorbia Kansui and Euphorbia Pekinensia on pregnant maintenances. They reported that Euphorbiae Kansui Radix and Euphonrbiae Pekinensis Radix obstruct pregnant maintenance and the functions of corpus lutein, give damages to the kidney and the liver during pregnancy.

Sipjotang are usually composed of four herbs such as Euphorbiae Kansui Radix, Daphinis Genkwa Flos, Euphonrbiae Pekinensis Radix (in identical ratio) and ten grains of Jujubae Fructus. Since the three of Euphorbiae Kansui Radix, Daphinis Genkwa Flos, Euphonrbiae Pekinensis Radix are poisonous and act in drastic way, it needs to add ten grains of Jujubae Fructus to buffer the toxicities and drastic action[2,4].

Generally, licorice is widely used to moderate other herbs' toxicities in Korean traditional medicine[14]. However, in this formula Jujubae Fructus is added to act as the toxicities' buffer since it is putatively regarded that the toxicities of Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix are augmented when combined with Licorice[2].

Additionally, there is another putative regarding that Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix must be administered in the form of powder other than in the form of decoction.

To evaluate the two putative regards, in this study 1. we compared the effects of three crude extracts from Euphorbiae Kansui Radix, Daphinis Genkwa Flos and Euphonrbiae Pekinensis Radix boiled in pure water, boiled in the medical solution obtained from boiling Jujuae fructus in pure water (details see methods) and boiled in the medical solution obtained from boiling licorice in pure water (details see methods) on rats' livers and kidneys. 2. We also compared the toxicity of powder form when administered together with water, the medical solution from Jujubae Fructus and licorice on rats' livers and kidneys respectively.

ALT and AST are the important enzymes associated with liver parenchymal cells, they raised when there is some damage in the liver. LDH is the enzyme that catalyses the conversion of pyruvate to lactate, exists in a wide variety of organisms, generally used to assess tissue breakdown. BUN test is the measure of the amount of nitrogen in blood, elevated BUN usually comes from poor function of the kidney[15]. In the present study, ALT, AST, LDH and BUN were employed to be biochemical markers for possible damage to the liver and the kidney caused by taking Sipjotang. The results in the present study showed that there is not any significant change in above tests compared to normal or control group. It means that 24 h after taking Sipjotang (in all doses used in this study) for 3 consecutive days, no significant biochemical changes were generated.

To further evaluate the effect of Sipjotang on liver and kidney, we conducted histological examining the samples from liver and kidney. A microscopic observation of parenchymal organs provides good information about organ morphology[16]. In the present study, no KWDD-treatment related histopathological changes were detected in rat's liver. However, increase of the number of tubules containing hyaline casts and atrophic tubules were observed in kidneys in all KWD decoction treated groups compared to that of normal or control group. The increase of abnormal changes in the kidney were inhibited by addition of Licorice or Jujubae Fructus extracts. Thus, the increase of abnormal tubules was considered as result of nephrotoxicity of KWDD, and it was effectively inhibited by addition of Licorice or Jujubae Fructus extracts. In addition, more palliated histopathological profiles were detected in Jujubae Fructus extract groups than Licorice extracts groups.

In the case of powder administered together with water, Jujubae Fructus extract or licorice extract, also no KWDP-treatment related histopathological changes in the liver were detected. Increase of tubules containing hyaline casts in kidney was still detected in KWDP2 group compared to that of normal or control group. There was no significant differences in the numbers of atrophic tubules observed in all powder groups compared to that of normal or control group (data not shown). The increase of abnormal changes on the kidney were inhibited by addition of Jujubae Fructus extracts (KWDPJ2) or Licorice (KWDPL2). Thus, the increase of tubules containing hyaline casts were considered as results of nephrotoxicity of taking high dose of KWDP. These nephrotoxicities were effectively inhibited by addition of Licorice or Jujubae Fructus extracts. Statistical analysis showed no significant difference between Jujubae Fructus groups and Licorice groups.

In generally, casts in kidney are cylindric protein accumulations which precipitate in the distal and collecting tubules of kidney. They are flushed from these areas and pass into the urine[17]. Several kinds of casts can be seen as possible indicators of renal diseases such as nephritis, pyelonephritis, amyloidosis or nephrosis[18]. Also these casts have been observed as signs of nephrotoxicity of chemical poisoning[19] and as a process of hyper-immune states[20], tumor[21] and ischemic renal diseases[22].

In the present study, hyaline casts are considered as results of nephrotoxicity of KWDD and KWDP treatment. These hyaline casts were decreased by Licorice or Jujubae Fructus extracts. Therefore, it was considered as evidence that Licorice and Jujubae Fructus extracts reduced the nephrotoxicity caused by KWDD and KWDP treatment.

In term of reducing the nephrotoxicty of KWDD, it is also considered that Jujubae Fructus extracts are more suitable than that of Licorice since more favorable effect was observed in Jujubae Fructus extracts groups in all doses used in the present study.

The focal tubular atrophy of kidney has been observed in various kidney diseases such as idiopathic membranous nephropathy[23], irradiation nephropathy[24], polyomavirus nephropathy[25], chemical induced nephropathies[26] and heavy metal poisoning[27], most of them were induced as a secondary or chronic process of abnormal glomerulus filtration and obstructive tubular passages[28,29]. Thus, the focal tubular atrophy detected in the present study considered as a secondary impact of hyaline casts that could interrupt the passage of urine through tubules, and it can also be considered as one signs of KWD-treatment related nephrotoxicity. Quite similar to those of hyaline casts, the numbers of atrophic tubules were decreased treated with extracts of Licorice or Jujubae Fructus in the study of decoction form. Especially, more favorable inhibitory effect were detected in Jujubae Fructus extracts groups (KWDDJs) than those of Licorice groups (KWDDLs) regardless of the doses used in the present study.

As mentioned above, there is a traditional regard that Sipjotang must be administered in the form of powder other than decoction mostly due to the possible toxicity brought by Sipjotang decoction. To assess this regard, the histopathological change caused by Sipjotang decoction and Sipjotang powder was compared. Statistical analysis showed that the powder form are more favorable than the decoction. Summing up, the results in the present study showed that: 1. In Sipjotang formula, Jujubae Fructus plays the role of abating toxicity of KWD, and in this aspect, is superior than general herbs' toxicity buffer Licorice. However the result of this study did not support that Licorice would increase the toxicity of KWD in Sipjotang formula. 2. Considering the potential bad effect of taking Sipjotang on kidney the form of powder of Sipjotang is more suitable to be taken than the form of decoction.

The results of present study experimentally support that Jujubae Fructus is more favorable in buffering KWD toxicity in Sipjotang formula than Licorice and the form of power is better than the form of decoction when taking Sipjotang. The underling mechanism of the effect of Jujubae Fructus on buffering KWD' toxicity and intensity needs further investigation.

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