Evaluation of Oriental medicines using a cultured renal epithelial cell line, LLC-PK_1 : Effects of Carthami Flos, Rhei Rhizoma and Astragali Radix

Takako YOKOZAWA,* Zhong Wu LIU and Cui Ping CHEN

Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University

(*Received June 26, 1998. Accepted September 25, 1998.*)

Abstract

The effects of Carthami Flos, Rhei Rhizoma and Astragali Radix extract, which have been proved to ameliorate renal failure in rats with glycerol-induced renal failure, were examined in cell culture under three different culture conditions. Rhei Rhizoma extract showed the most potent effect among the three crude drugs under any of the routine, hypoxia-reoxygenation and cisplatin exposure conditions employed, suggesting that its favorable effect on proximal tubule function is due to suppression of lipid peroxidation *via* free radicals. In contrast, this anti-cytotoxic activity was low in Astragali Radix extract, and almost nil in Carthami Flos extract. These results indicate that Rhei Rhizoma, Astragali Radix and Carthami Flos exert their actions on different sites in the kidney.

Key words Rhei Rhizoma, Astragali Radix, Carthami Flos, renal injury, LLC-PK_1, lactate dehydrogenase, malondialdehyde.

Introduction

Acute tubular necrosis, which is the most common pathological change in cases of acute renal failure, has been shown to result from renal ischemia and the effects of nephrotoxic substances. Although renal ischemia and nephrotoxicity differ from each other in their pathogenesis and the mechanism by which they induce acute tubular necrosis, tubular necrosis is the final histological feature in both conditions.[1] Recently, the possibility that apoptosis is involved in the acute renal failure characterized by acute tubular necrosis has been pointed out by Beeri *et al.*,[2] Lieberthal *et al.*[3] and Takeda *et al.*[4]

Currently available drugs effective for prevention and treatment of acute renal failure are very limited. Therapeutic policies for this condition in Chinese medicine are to use drugs that promote blood circulation and remove blood stasis in the initial stage after onset, cathartics and diuretics in the oliguric stage, and invigorating drugs in the polyuric and convalescent stages.[5,6] We have previously examined the actions of three representative Oriental medicines, i.e., Carthami Flos (a drug for promoting blood circulation and removing blood stasis), Rhei Rhizoma (a cathartic) and Astragali Radix (an invigorating drug), in rats with glycerol-induced acute renal failure, an animal model which includes both ischemic and nephrotoxic factors and is believed to be closest to human acute renal failure. In rats given Carthami Flos, Rhei Rhizoma or Astragali Radix extracts, there was a decrease in the levels of urea nitrogen and creatinine (Cr) in serum, and the fractional excretion of sodium (FE_{Na}). In addition, Carthami Flos extract caused an increase in urinary osmotic pressure, Rhei Rhizoma extract caused a decrease in urine volume, and Astragali Radix extract caused an increase in creatinine clearance (C_{Cr}), suggesting that these drugs ameliorate the condition of acute renal failure in different ways.[7] On the other hand, the histopathological features of acute renal failure include cast

*〒930-0194 富山市杉谷2630
富山医科薬科大学和漢薬研究所　横澤隆子
2630 Sugitani, Toyama 930-0194, Japan

formation in the renal tubules and exfoliation and necrosis of epithelial cells in the proximal tubules, indicating the presence of injury to tubule epithelial cells.[8]

However, the kidney is an organ consisting of various types of cells, and therefore, there are limits to the investigation of biological changes in tubule epithelial cells if the whole kidney is involved. In recent years there has been progress in techniques of cell culture and the establishment of various cultured cell lines derived from the kidney, which maintain the characteristics of the proximal tubule, distal tubule or collecting tubule. Proximal tubules are known to be severely injured in acute renal failure.[9]

In the present study, using a swine kidney-derived cultured epithelial cell line LLC-PK_1, which retains the nature of the proximal tubule, we evaluated the effects of the three Oriental medicines-Carthami Flos, Rhei Rhizoma and Astragali Radix-proved to be effective in a previous study using rats with glycerol-induced renal failure.

Materials and Methods

Medium and reagents : Dulbecco's modified Eagle medium/nutrient mixture F-12 (D-MEM/F-12) and fetal calf serum (FCS) were purchased from Life Technologies, Inc. (Grand Island, NY, USA) and Cell Culture Laboratories (Cleveland, OH, USA), respectively. Cisplatin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). A commercial kit (lactate dehydrogenase CII-Test) for assaying lactate dehydrogenase was obtained from Wako Pure Chemical Industries, Ltd., Osaka, Japan.

Crude drugs : The crude drugs used in this experiment were Carthami Flos (*Carthamus tinctorius* L.), Rhei Rhizoma (*Rheum officinale* BAILLON) and Astragali Radix (*Astragalus membranaceus* BUNGE). One hundred grams of each crude drug was boiled gently in 1,000 ml of water for 60 min. The extract was then concentrated under reduced pressure to leave a residue. The yields of Carthami Flos, Rhei Rhizoma and Astragali Radix were 31 %, 22 % and 27 %, respectively.

Lactate dehydrogenase (LDH) and thiobarbituric acid (TBA)-reactive substance leakage assays : Commercially available LLC-PK_1 cells were maintained at 37°C in a humidified atmosphere of 5 % CO_2 in air (routine conditions) in culture plates (Corning Glass Works, Corning, NY) with 5 % FCS-supplemented D-MEM/F-12 medium. After confluence had been reached, 10^4 cells per well were seeded in fresh 96-well culture plates, the required extract and/or cisplatin (0.25 μM) was added to the culture medium 2 h later, and the plates were incubated under routine conditions for 48 h. In another experiment, the cells were cultured under routine conditions for 41 h, then subjected to hypoxia in an anaerobic chamber for 6 h and returned to routine conditions for the final 1 h. Leakage of LDH into the culture medium, an index of cytotoxicity, was assayed using a commercial kit, and the extent of lipid peroxidation was estimated by measuring the concentration of TBA-reactive substance in the medium, as described by Yagi.[10]

Statistics : All the values presented are means ± S.E. of 5 determinations and, where appropriate, the significance of differences was tested using Dunnett's method. Differences at p values of less than 0.05 were considered statistically significant.

Results

LDH leakage from cultured cells

During routine incubation, a certain amount of LDH (about 117.9 mU/ml) was released from the cells, as shown in Table I. LDH release by cells cultured with the extract of Rhei Rhizoma was reduced concentration-dependently, whereas the Astragali Radix extract exerted only limited effects at relatively high concentrations, and the effect of Carthami Flos was very weak. When the cells were subjected to hypoxia for 6 h (in an anaerobic incubator), followed by routine culture for a further 1 h, leakage of LDH from the control cells increased sharply to about 128 % of the normal value, as shown in Table I. However, although LDH leakage was still higher than that of the corresponding normal cultures, it was reduced dramatically by culturing the cells with a low concentration of Rhei Rhizoma (2.5 μg/ml) extract. Higher concentrations of Astragali Radix and Carthami Flos extracts (10 times and 20 times those of Rhei Rhizoma,respectively) were required to

Table I Effect of Carthami Flos, Rhei Rhizoma and Astragali Radix on LDH leakage from LLC-PK$_1$ cells.

Group	Concentration (μg/ml)	LDH activity (mIU/ml)		
		Routine	Hypoxia/reoxygenation	Cisplatin
Carthami Flos	2.5	116.4 ± 4.9	147.6 ± 4.2	241.2 ± 8.1
	12.5	112.1 ± 4.7	143.8 ± 5.1	243.5 ± 7.8
	25	110.7 ± 4.5	142.9 ± 5.8	239.8 ± 6.3
	50	109.9 ± 5.6	138.1 ± 4.7^b	236.0 ± 7.3
	125	108.9 ± 4.9^a	132.0 ± 3.5^c	234.2 ± 7.1
Rhei Rhizoma	2.5	111.0 ± 3.7	140.0 ± 4.3^a	238.8 ± 6.5
	12.5	102.8 ± 4.3^c	136.2 ± 4.1^b	230.9 ± 5.0^b
	25	94.6 ± 3.6^c	133.3 ± 5.4^c	226.2 ± 6.3^c
	50	90.7 ± 5.1^c	127.7 ± 3.6^c	215.2 ± 4.8^c
	125	82.2 ± 4.8^c	118.5 ± 5.2^c	212.3 ± 4.6^c
Astragali Radix	2.5	115.3 ± 4.5	146.7 ± 6.7	244.6 ± 5.6
	12.5	112.3 ± 4.5	143.9 ± 5.9	240.9 ± 4.8
	25	109.0 ± 5.2^a	139.6 ± 5.1^a	235.9 ± 6.7
	50	100.3 ± 4.9^c	132.3 ± 3.7^c	231.2 ± 5.1^a
	125	96.8 ± 4.8^c	127.5 ± 4.2^c	222.3 ± 5.6^c
	−	117.9 ± 3.4	150.6 ± 5.5	244.2 ± 5.1

Significantly different from the non-additive value: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$.

inhibit LDH release to the same extent. As shown in Table I, LDH leakage from the cells into the culture medium increased after cisplatin had been added to the medium. Although there was no suppression of the cytotoxic effect of cisplatin in the presence of 2.5 μg/ml Rhei Rhizoma, 12.5 μg/ml suppressed the leakage significantly to 230.9 mIU/ml. This suppression became more marked as the concentration of Rhei Rhizoma increased; the leakage of LDH into the culture medium was found to be suppressed in a dose-dependent manner. Astragali Radix exerted only a limited effect at relatively high concentrations, whereas Carthami Flos did not affect LDH release.

TBA-reactive substance level released into the culture medium

In routine culture medium, 0.109 nmol of TBA-reactive substance was detected in the control group, and the amount of this lipid peroxidant formed declined markedly when the cells were cultured in the presence of extracts of Rhei Rhizoma and Astragali Radix. Their inhibition of TBA-reactive substance formation was concentration-dependent. Among the 5 concentrations tested, 2.5 μg/ml Rhei Rhizoma reduced TBA-reactive substance production significantly, 10 times of the concentration of Astragali Radix extract was required for the same effect, but Carthami Flos extract had no such effect on TBA-reactive substance release, as shown in Table II. Exposure to hypoxia for 6 h followed by reoxygenation for 1 h increased the TBA-reactive substance level in the control cell medium sharply, to about 1.39 times the intact control value. The TBA-reactive substance level was reduced to 76 % of the hypoxic-reoxygenated control value by 125 μg/ml Rhei Rhizoma extract. When LLC-PK$_1$ cells were cultured with Astragali Radix extract at graded concentrations ranging from 25 to 125 μg/ml, the TBA-reactive substance release patterns were similar to those observed for LDH leakage. However, Carthami Flos extract had no such effect on TBA-reactive substance release. A similar effect was found in cells exposed to cisplatin; a large amount of TBA-reactive substance leakage, up to about 1.6 times that observed in the routine blank, was observed. When the cells were cultured under identical conditions except for the dose of Rhei Rhizoma extract, this leakage was substantially inhibited in a concentration-dependent manner. The inhibition of this event in the Rhei Rhizoma extract-treated group resembled that for LDH, i.e. cells treated with a concentration ranging from 12.5 to

Table II Effect of Carthami Flos, Rhei Rhizoma and Astragali Radix on TBA-reactive substance leakage from LLC-PK_1 cells.

Group	Concentration (μg/ml)	TBA-reactive substance (nmol/ml)		
		Routine	Hypoxia/reoxygenation	Cisplatin
Carthami Flos	2.5	0.109 ± 0.010	0.152 ± 0.004	0.171 ± 0.008
	12.5	0.106 ± 0.007	0.149 ± 0.006	0.167 ± 0.006
	25	0.106 ± 0.005	0.144 ± 0.006	0.165 ± 0.007
	50	0.104 ± 0.005	0.145 ± 0.007	0.165 ± 0.006
	125	0.102 ± 0.008	0.143 ± 0.005	0.163 ± 0.007
Rhei Rhizoma	2.5	0.096 ± 0.004[b]	0.142 ± 0.005[a]	0.165 ± 0.007
	12.5	0.091 ± 0.005[c]	0.129 ± 0.005[c]	0.155 ± 0.006[c]
	25	0.085 ± 0.006[c]	0.118 ± 0.004[c]	0.143 ± 0.005[c]
	50	0.077 ± 0.006[c]	0.112 ± 0.004[c]	0.135 ± 0.003[c]
	125	0.067 ± 0.005[c]	0.115 ± 0.004[c]	0.123 ± 0.005[c]
Astragali Radix	2.5	0.109 ± 0.007	0.149 ± 0.005	0.170 ± 0.007
	12.5	0.103 ± 0.005	0.146 ± 0.004	0.165 ± 0.006
	25	0.095 ± 0.004[b]	0.138 ± 0.005[b]	0.157 ± 0.004[b]
	50	0.091 ± 0.005[c]	0.131 ± 0.005[c]	0.146 ± 0.007[c]
	125	0.085 ± 0.007[c]	0.125 ± 0.004[c]	0.138 ± 0.006[c]
	−	0.109 ± 0.006	0.151 ± 0.005	0.174 ± 0.005

Significantly different from the non-additive value: [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$.

125 μg/ml were significantly affected. However, a higher concentration of Astragali Radix and Carthami Flos extracts was required to inhibit TBA-reactive substance release (Table II).

Discussion

Rats with glycerol-induced renal failure are considered to be the best animal model of human acute renal failure, both ischemic and nephrotoxic factors being involved.[11-15] Among the three different Oriental drugs examined, Astragali Radix and Carthami Flos extracts showed common actions on urea nitrogen and Cr in serum and Ccr, with some differences in potency. Astragali Radix extract, however, had an influence on Ccr, while Carthami Flos extract acted on urinary osmotic pressure. These findings suggest that Astragali Radix and Carthami Flos exert their actions on glomeruli and renal tubules, respectively. All three drugs tested also caused a decrease in the FE_{Na} level (as calculated from Na and Cr levels in blood and urine), which is elevated under the condition of acute renal failure, suggesting that the mutual functions of the renal tubules and glomeruli were improved. Under the usual conditions of renal epithelial cell culture (95 % air, 5 % CO_2), the leakage of LDH, a lysosomal enzyme, and TBA-reactive substance released into the culture medium by cell membrane injury, was significantly suppressed in the presence of Rhei Rhizoma extract, without any influence on the viability of the cells. However, such a suppressive effect of the LDH and TBA-reactive substance leakage was only slight with Astragali Radix extract, and practically nil with Carthami Flos extract.

On the other hand, the leakage of LDH and TBA-reactive substance into the culture medium was markedly increased by reoxygenation after 6-h incubation of LLC-PK_1 cells under hypoxic conditions. In the presence of Rhei Rhizoma extract, however, the leakage of the enzyme and lipid peroxides was significantly suppressed, showing a dose-dependent enhancement of the effect. Yonehana and Gemba[16] found in their experiments using LLC-PK_1 cells that the intracellular level of the antioxidant glutathione was significantly decreased under hypoxic conditions. Snowdowne *et al.*[17] and Kribben *et al.*[18] reported that hypoxia caused an increase in intracellular Ca in a kidney-derived culture cell line, LLC-MK_2, and also in isolated renal tubules. On the other hand, with regard to the cytotoxicity of reoxygenation, Paller *et*

al.,[19] in 1984, demonstrated the involvement of active oxygen in the pathogenesis of ischemia-reperfusion injury in the kidney. The introduction of this novel view by their report had a large influence on research in this area, and subsequent studies revealed a close relationship between ischemia-reperfusion injury and diseases of various organs. We have reported previously that, when the proximal tubule-like cultured cell line LLC-PK_1, which was also used in the present study, was cultured in the presence of the hydroxyl radical (·OH) scavenger N,N'-dimethyl thiourea, leakage of LDH and TBA-reactive substance was suppressed.[20] We explained this phenomenon in terms of the cytotoxicity of free radicals produced by renal epithelial cells through hypoxia and reoxygenation. This speculation is consistent with the report by Paller *et al.*,[21] who observed the production of free radicals by renal epithelial cells in primary cultures of renal cells. The suppressive effect of Rhei Rhizoma extract on this cytotoxicity provides *in vitro* evidence which corroborates our observation of the effect of oral administration of Rhei Rhizoma extract in rats with renal failure under oxidative stress,[22-24] and demonstrates the direct effect of Rhei Rhizoma extract on renal cells.

Cisplatin is a platinum chelate which exerts a potent antitumor action on cancers of the testis, ovary, urinary bladder, prostate, and head and neck. On the other hand, it has adverse effects on the kidney, bone marrow and digestive organs ; the risk of nephropathy, in particular, is so large as to limit the use of this agent.[25-28] Since the major site of action of cisplatin in the kidney is the straight part of the proximal tubule, we examined the effect of cisplatin on LLC-PK_1 cells. We found that the release of LDH was increased to a level about 2.5 times higher than that in the absence of cisplatin, demonstrating the cytotoxicity of this agent. In contrast, when Rhei Rhizoma extract was added to the culture medium at graded concentrations, the release of LDH was decreased as the concentration of Rhei Rhizoma extract increased, showing that Rhei Rhizoma decreased the cytotoxicity of cisplatin. With regard to the mechanism of cisplatin-induced nephropathy, Sugihara *et al.*[29] and Hannemann and Baumann[30] found in experiments using rat renal cortical sections that cisplatin caused an increase in the production of lipid peroxides and dysfunction of renal cells. On the other hand, McGinness *et al.*[31] reported that cisplatin-induced nephropathy was reduced by superoxide dismutase, and Baliga *et al.*[32] reported a similar reduction by the ·OH scavenger N,N'-dimethyl thiourea. In the present study, cisplatin also caused a marked increase in the leakage of LDH and TBA-reactive substance into the culture medium. However, when Rhei Rhizoma extract was present, the leakage of TBA-reactive substance was significantly suppressed, indicating the cell-protective effect of Rhei Rhizoma. Since Rhei Rhizoma extract also reduced the cytotoxicity under hypoxia-reoxygenation conditions, its favorable effect on proximal tubule function was attributed to the suppression of lipid peroxidation *via* free radicals.

On the other hand, the anti-cytotoxic activity of Astragali Radix extract was lower than that of Rhei Rhizoma extract under any of the routine, hypoxia-reoxygenation and cisplatin exposure conditions employed. In a previous study, Astragali Radix extract reduced the levels of urea nitrogen and Cr in serum and Ccr in rats with glycerol-induced renal failure, showing a potent effect on hyperazotemia.[7] Therefore, it was speculated that Astragali Radix exerted a favorable effect on glomerular function through its action on the nitrogen metabolism system in the body, leading to amelioration of renal failure, rather than exerting a direct effect on renal cells.

Although Carthami Flos extract markedly improved urine osmotic pressure in rats with glycerol-induced renal failure, it had no significant effect on LLC-PK_1 cells, suggesting that its site of action is elsewhere, e.g., the distal tubule or collecting tubule.

Thus, this experiment using renal cells revealed that Rhei Rhizoma, Astragali Radix and Carthami Flos extracts exert their actions on different sites in the kidney, although all three improve the state of acute renal failure induced by glycerol.

和文抄録

グリセロール誘発腎不全ラットにおいて，腎不全状態の是正を示した大黄，黄耆，紅花の近位尿細管様細胞のLLC-PK_1を用い，3種類の培養条件下で比較検討した。

大黄エキスは通常，低酸素－再酸素，cisplatin 暴露培養条件下いずれにおいてもその活性は3種類の中で最も強く，フリーラジカルを介した脂質過酸化抑制作用が近位尿細管機能に好影響をもたらしたものと推測されたが，そのような作用は黄耆では弱く，紅花ではほとんど認められなかった。このことから腎不全ラットで有効性を示した大黄，黄耆，紅花はそれぞれ異なった部位で作動しているものと考えられた。

References

1) Rosen, S., Brezis, M. and Stillman, I.: The pathology of nephrotoxic injury: A reappraisal. *Miner. Electrolyte Metab.* **20**, 174-180, 1994.
2) Beeri, R., Symon, Z., Brezis, M., Ben-Sasson, S.A., Baehr, P.H., Rosen, S. and Zager, R.A.: Rapid DNA fragmentation from hypoxia along the thick ascending limb of rat kidneys. *Kidney Int.* **47**, 1806-1810, 1995.
3) Lieberthal, W., Triaca, V. and Levine, J.: Mechanisms of death induced by cisplatin in proximal tubular epithelial cells: apoptosis vs. necrosis. *Am. J. Physiol.* **270**, F700-F708, 1996.
4) Takeda, M., Kobayashi, M., Shirato, I., Osaki, T. and Endou, H.: Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1β converting enzyme (ICE) family of proteases but inhibited by overexpression of Bcl-2. *Arch. Toxicol.* **71**, 612-621, 1997.
5) Cao, X.: Clinical study on traditional Chinese medicine treatment of acute renal failure. *J. Trad. Chin. Med.* **29**, 54-57, 1988.
6) Chen, M.: Diagnosis and treatment of acute renal failure. *Chin. J. Integr. Med.* **7**, 45-47, 1987.
7) Yokozawa, T., Liu, Z.W., Shibata, T. and Hasegawa, M.: Role of Oriental medicines in the treatment of acute renal failure: Carthami Flos, Rhei Rhizoma and Astragali Radix. *J. Trad. Med.* **14**, 49-53, 1997.
8) Racusen, L.C.: The histopathology of acute renal failure. *New Horizons* **3**, 662-668, 1995.
9) Gstraunthaler, G.J.: Epithelial cells in tissue culture. *Renal Physiol. Biochem.* **11**, 1-42, 1988.
10) Yagi, K.: A simple fluorometric assay for lipoperoxide in blood plasma. *Biochem. Med.* **15**, 212-216, 1976.
11) Oken, D.E., Arce, M.L. and Wilson, D.R.: Glycerol-induced hemoglobinuric acute renal failure in the rat. *J. Clin. Invest.* **45**, 724-735, 1966.
12) Stein, J.H., Lifschitz, M.D. and Barnes, L.D.: Current concepts on the pathophysiology of acute renal failure. *Am. J. Physiol.* **234**, F171-F181, 1978.
13) Thiel, G., Wilson, D.R., Arce, M.L. and Oken, D.E.: Glycerol-induced hemoglobinuric acute renal failure in the rat. *Nephron* **4**, 276-297, 1967.
14) Hishida, A., Kato, A. and Yamada, M.: Myoglobinuria and acute renal failure. *Jpn. J. Clin. Pathol.* **39**, 110-114, 1991.
15) Ishikawa, I.: Myohemoglobin-induced acute renal failure. *Kidney and Dialysis* **31**, 146-150, 1991.
16) Yonehana, T. and Gemba, M.: Hypoxia and reoxygenation-induced injury of renal epithelial cells: Effect of free radical scavengers. *Jpn. J. Pharmacol.* **68**, 231-234, 1995.
17) Snowdowne, K.W., Freudenrich, C.C. and Borle, A.B.: The effects of anoxia on cytosolic free calcium, calcium fluxes, and cellular ATP levels in cultured kidney cells. *J. Biol. Chem.* **260**, 11619-11626, 1985.
18) Kribben, A., Wieder, E.D., Wetzels, J.F.M., Yu, L., Gengaro, P.E., Burke, T.J. and Schrier, R.W.: Evidence for role of cytosolic free calcium in hypoxia-induced proximal tubule injury. *J. Clin. Invest.* **93**, 1922-1929, 1994.
19) Paller, M.S., Hoidal, J.R. and Ferris, T.F.: Oxygen free radicals in ischemic acute renal failure in the rat. *J. Clin. Invest.* **74**, 1156-1164, 1984.
20) Yokozawa, T., Oura, H. and Nishioka, I.: Confirmation that magnesium lithospermate B ameliorates paraquat-induced injury in cultured renal epithelial cells. *Nephron* **79**, 373-374, 1998.
21) Paller, M.S. and Neumann, T.V.: Reactive oxygen species and rat renal epithelial cells during hypoxia and reoxygenation. *Kidney Int.* **40**, 1041-1049, 1991.
22) Yokozawa, T., Suzuki, N., Zheng, P.D., Oura, H. and Nishioka, I.: Effect of orally administered rhubarb extract in rats with chronic renal failure. *Chem. Pharm. Bull.* **32**, 4506-4513, 1984.
23) Yokozawa, T., Suzuki, N., Okuda, I., Oura, H. and Nishioka, I.: Uremia-preventive effect of rhubarb extract in rats. *J. Med. Pharm. Soc. WAKAN-YAKU* **2**, 344-350, 1985.
24) Yokozawa, T., He, L.Q., Muto, Y., Nagasaki, R., Hattori, M. and Oura, H.: Effects of rhubarb extract in rats with diabetic nephropathy. *Phytother. Res.* **11**, 73-75, 1997.
25) Blachley, J.D. and Hill, J.B.: Renal and electrolyte disturbance associated with cisplatin. *Ann. Intern. Med.* **95**, 628-632, 1981.
26) Goldstein, R.S. and Mayor, G.H.: The nephrotoxicity of cisplatin. *Life Sci.* **32**, 685-690, 1983.
27) Weiner, M.W. and Jacobs, C.: Mechanism of cisplatin nephrotoxicity. *Federation Proc.* **42**, 2974-2978, 1983.
28) Litterst, C.L.: Cisplatinum: A review, with special reference to cellular and molecular interactions. *Agents and Actions* **15**, 520-524, 1984.
29) Sugihara, K., Nakano, S. and Gemba, M.: Effect of cisplatin on *in vitro* production of lipid peroxides in rat kidney cortex. *Jpn. J. Pharmacol.* **44**, 71-76, 1987.
30) Hannemann, J. and Baumann, K.: Cisplatin-induced lipid peroxidation and decrease of gluconeogenesis in rat kidney cortex: Different effects of antioxidants and radical scavengers. *Toxicology* **51**, 119-132, 1988.
31) McGinness, J.E., Proctor, P.H., Demopoulos, H.B., Hokanson, J.A. and Kirkpatrick, D.S.: Amelioration of cis-platinum nephrotoxicity by orgotein (superoxide dismutase). *Physiol. Chem. Phys.* **10**, 267-277, 1978.
32) Baliga, R., Zhang, Z.W., Baliga, M., Ueda, N. and Shah, S.V.: *In vitro* and *in vivo* evidence suggesting a role for iron in cisplatin-induced nephrotoxicity. *Kidney Int.* **53**, 394-401, 1998.