

Protective effects of cystone, a polyherbal ayurvedic preparation, on cisplatin-induced renal toxicity in rats

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Abstract

Cystone, a polyherbal ayurvedic preparation, was found to protect rats partially but significantly against cisplatin-induced renal toxicity, when given intraperitoneally 1 h before cisplatin. At 500 and 1000 $\mu\text{g/ml}$, it also inhibited lipid peroxidation induced by cisplatin in renal cortical slices by 62.7 and 71.6%, respectively. The rats pretreated with cystone (1000 mg/kg i.p.) had significantly lower blood urea nitrogen (BUN) and serum creatinine (33.8 and 0.92 mg/dl, respectively) compared to cisplatin alone (51.5 and 1.41 mg/dl, respectively). The control animals had 17.1 and 0.63 mg/dl, respectively. The cystone treated animals lost 5.63 g body weight compared to 12.5 g for cisplatin alone treated animals on day 5. Renal functions like urine to serum creatinine ratio and creatinine clearance showed significant improvement when cystone was given 1 h before cisplatin. However, cystone did not protect increased excretion of urinary protein and decreased WBC count caused by cisplatin. The present study suggests that the cystone protects kidney against cisplatin-induced toxicity and the protection may be mediated through its ability to inhibit lipid peroxidation. © 1998 Published by Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cisplatin; Renal toxicity; Cystone; Lipid peroxidation; Serum creatinine; Urea nitrogen

1. Introduction

Cystone is an ayurvedic polyherbal preparation containing herbal extracts and minerals (Table 1). The plants used in the preparation are well known in the ayurvedic medicine for their beneficial actions on the kidney (Nadkarni, 1992). Cystone

has been clinically used extensively for treating many urinary tract complications such as urolithiasis (Rai, 1960), burning micturition (Garg and Singh, 1985), neuro-ureterolithiasis (Misgar, 1982), urinary tract complications in pregnancy (Sengupta, 1987) and other various renal disorders (Sharma et al., 1983).

Cisplatin is a potent anticancer agent used in solid tumors of testes, ovary, breast, lungs, bladder etc. (Rozenewieg et al., 1977). Its clinical use

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Table 1
Composition of cystone^a

Plant name	Family	Part used	Quantity (mg)
<i>Didymocarpus pedicellata</i> R.Br.	Gesneriaceae	Flower	65
<i>Saxifraga ligulata</i> Walld.	Saxifragaceae	Stem	49
<i>Rubia cordifolia</i> L.	Rubiaceae	Stem	16
<i>Cyperus scariosus</i> R.Br.	Cyperaceae	Root	16
<i>Achyranthes aspera</i> L.	Amaranthaceae	Whole plant	16
<i>Onosma bracteatum</i> Walld.	Boraginaceae	Whole plant	16
<i>Vernonia cinerea</i> L.	Compositae	Whole plant	16
Shilajeet (purified)	Bituminous material oozing from rock in summer		13
Hajrul Yahood Bhasma	Fossil stone occurring as a petrified oblong pointed fruit		16

Processed with *Ocimum basilicum* L. (Labiatae), *Tribulus terrestris* L. (Zygophyllaceae), *Mimosa pudica* L. (Mimosaceae), *Dolichos biflorus* L. (Papilionaceae), *Pavonia odorata* Willd. (Malvaceae), *Equisetum arvense* L. (Equisetaceae) and *Tectona grandis* L.f. (Verbenaceae).

^a Cystone is a ayurvedic formulation prepared and marketed in India.

is limited by its renal toxicity (Madias and Harrington, 1978; Goldstein and Mayor, 1983). Although the mechanism of action of the renal toxicity of cisplatin is still not clear, it has been suggested that the oxygen free radicals play an important role. Cisplatin is known to cause increased lipid peroxidation in renal cortical slices. (Inselmann et al., 1995). Many antioxidants such as sodium selenite (Baldew et al., 1989), hydroxy ethyl rutoside (Bull et al., 1988) etc, are known to reduce the renal toxicity caused by cisplatin. The crude extract of *Ginkgo biloba* L. (Family: Ginkgoaceae) protects kidney slices against cisplatin-induced lipid peroxidation and decreased uptake of *p*-aminohippuric acid (Inselmann et al., 1995). Our preliminary studies showed that cystone inhibited the lipid peroxidation in renal cortical slices induced by cisplatin. Since, cystone is clinically used in various renal dysfunctions, we found it interesting to further investigate the effect of cystone on the renal toxicity induced by cisplatin.

2. Materials and methods

2.1. Animals

Male Wistar rats of 8–10 week of age, weighing 200–230 g were used for the study. They were maintained on a standard diet (Lipton's India, Calcutta) and water was given ad libitum.

2.2. Drug solutions

To 1000 mg cystone powder (Himalaya Drug, Bangalore), 10 ml distilled water was added and kept overnight at room temperature ($25 \pm 2^\circ\text{C}$) followed by boiling for 5 min. After cooling, the extract was filtered and the volume was made up to 10 ml. A separate experiment showed that 1000 mg of cystone powder yielded 210 mg of water soluble extract. The filtrate (equivalent 100 mg/ml to cystone powder) was used for the study.

Cisplatin (Sigma, St. Louis, MO) was dissolved in distilled water to give 1 mg/ml solution and prepared freshly.

2.3. Lipid peroxidation in rat renal cortical slices

Lipid peroxidation in renal cortical slices was measured according to the method as reported previously (Yamasaki et al., 1996). Immediately after the rats had been decapitated, the kidneys were removed and renal cortical slices were prepared using a razor blade in a kidney holder device to achieve a slice thickness of about 0.3–0.5 mm. Kidney slices of about 100 mg/sample were incubated in buffer consisting of (in mM): NaCl (137), KCl (5.9), CaCl₂ (1.5), MgCl₂ (1.2), glucose (11.5), 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (5.8), pH adjusted to 7.4 with NaOH.

Kidney slices were incubated in a sample volume of 4 ml with cisplatin concentrations of 0.5, 1.0, 2.0 and 4.0 mM in the presence or absence of different concentrations of cystone. Incubations were carried out for 120 min at 37°C. Controls were incubated under identical conditions without addition of cisplatin. At the end of incubations, renal cortical slices were taken from each sample to determine the amount of malondialdehyde (MDA) as indicator of lipid peroxidation. The kidney slices were quickly removed from the medium, blotted on filter paper, weighed and homogenized in 5 ml sodium phosphate buffer (pH 7.4) at 2°C, centrifuged at 1000 g for 10 min at 2°C. To 1 ml supernatant, 1 ml thiobarbituric acid reagent (containing 0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 N hydrochloric acid) was added and heated at 90°C for 45 min, cooled and absorbance was measured at 532 nm. The amount of lipid peroxidation was expressed as nmol of (MDA) using the molar extinction coefficient of $1.56 \times 10^5/\text{M}$ per cm as reported earlier by us (Rajakumar and Rao, 1993).

2.4. Renal toxicity

Six groups of animals ($n = 8$) were investigated. For Group 1 animals, cisplatin solution was given at a dose of 3 mg/kg intraperitoneally (i.p.). Group 2, 3, 4 and 5 animals received cystone 100, 250, 500 and 1000 mg/kg, i.p. or orally 1 h before cisplatin 3 mg/kg, i.p., respectively. Group 3 acted as vehicle control (distilled water). The body weight was recorded daily and on day 5, the blood was collected from retroorbital venous plexus to measure blood urea nitrogen (BUN), serum creatinine, total proteins and WBC count. BUN and serum creatinine were measured using commercial kits (Ranbaxy Diagnostics, New Delhi, India). WBC count was carried out using a hemocytometer. Serum protein was measured by Lowry's method (Lowry et al., 1951). Urine was collected on day 5 for 6 h (initiated at 8 a.m.) using metabolic cages and analysed for creatinine and protein (Godkar, 1994).

2.5. Statistical analysis

Values are expressed as mean \pm S.E.M. For comparison, ANOVA followed by Student's Newman-Keuls test was used. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effect on lipid peroxidation

Incubation of renal cortical slices for 120 min with cisplatin resulted in increased MDA levels and this effect was found to be concentration dependent (Table 2). Addition of cystone was found to protect cortical slices against cisplatin-induced lipid peroxidation (Table 2). Cystone at 100, 500 and 1000 $\mu\text{g}/\text{ml}$ inhibited the lipid peroxidation induced by cisplatin (2.0 mM) by 41.8, 62.7 and 71.6%, respectively.

Table 2
Effect of cystone on cisplatin-induced lipid peroxidation in rat renal cortical slices

Treatment ^a	MDA (nmol/ mg protein)	Inhibition (%)
Control	0.51 \pm 0.04	
Cisplatin (in mM)		
0.5	0.61 \pm 0.05	
1.0	0.78 \pm 0.05*	
2.0	1.18 \pm 0.09*	
4.0	1.36 \pm 0.12*	
Cisplatin 2.0 mM plus cystone (in $\mu\text{g}/\text{ml}$)		
50	1.09 \pm 0.10*	13.4
100	0.90 \pm 0.07* #	41.8
500	0.76 \pm 0.08* #	62.7
1000	0.70 \pm 0.06*	71.6

^a Renal cortical slices were incubated with cisplatin and cystone for 120 min. MDA was measured as nmol per mg of tissue protein and expressed as mean \pm S.E.M. ($n = 4$). Percentage of inhibition was calculated and compared to cisplatin 2.0 mM.

* $P < 0.05$ compared to control.

$P < 0.05$ compared to cisplatin 2.0 mM.

Table 3
Effect of cystone on the elevation of BUN and serum creatinine induced by cisplatin

Treatment ^a	BUN (mg/dl)	Serum creatinine (mg/dl)
Control	17.1 ± 1.14	0.63 ± 0.04
Cisplatin, 3 mg/kg	51.5 ± 2.98*	1.41 ± 0.11*
Cisplatin, 3 mg/kg plus cystone		
100 mg/kg	52.0 ± 3.18*	1.43 ± 0.13*
250 mg/kg	48.1 ± 2.07*	1.39 ± 0.17*
500 mg/kg	44.5 ± 3.20*	1.30 ± 0.15*
1000 mg/kg	33.8 ± 2.60* #	0.92 ± 0.10* #

^a Cisplatin was given i.p. and cystone was given i.p. 1 h before cisplatin. After 5 days blood was collected and estimated for BUN and serum creatinine. Values are expressed as mean ± S.E.M. (n = 8).

* P < 0.05 compared to control.

P < 0.05 compared to cisplatin treated.

3.2. Effect on the body weight of rats

When animals were given cisplatin 3 mg/kg i.p., the weight of the animals decreased significantly. There was an average decrease of 12.5 ± 1.6 g on day 5 compared to control animals which gained 8.13 ± 1.3 g during the same period. When the animals were given cystone 100, 250 and 500 mg/kg i.p. 1 h before cisplatin 3 mg/kg i.p., the decrease in the body weight on day 5 was not reversed significantly. Upon administration of cystone at 1000 mg/kg i.p., 1 h before cisplatin 3 mg/kg i.p., the decrease in the body weight on day 5 was found to be only 5.63 ± 3.3 g. Thus, cystone at 1000 mg/kg i.p. was able to protect the animals against cisplatin-induced decrease in body weight. However, the protection was not complete.

3.3. Effect on BUN and serum creatinine

Administration of cisplatin 3 mg/kg i.p., resulted in significant increase in BUN and serum creatinine compared to control animals (Table 3). When cystone at 100, 250 and 500 mg/kg i.p. was given 1 h before cisplatin, the BUN levels did not alter. Cystone at 1000 mg/kg i.p. 1 h before cisplatin i.p., showed about 50% protection against the elevation of BUN. Similar results were

obtained with serum creatinine. When cisplatin was given, the serum creatinine level was increased significantly compared to control animals. Cystone at 100, 250 and 500 mg/kg i.p. did not protect increased SC levels induced by cisplatin 3 mg/kg i.p. (Table 3). When cystone 1000 mg/kg i.p. was given 1 h before cisplatin, about 62% protection against the elevation of serum creatinine was shown.

Thus, the nephrotoxicity of cisplatin characterized by the elevation of BUN and serum creatinine was reversed to a significant extent by cystone at 1000 mg/kg i.p.

3.4. Effect on renal functions

The deterioration of the renal functions induced by cisplatin and the effect of cystone is given in Table 4. There was a slight reduction in urine volume when cisplatin was given compared to control animals. Cystone did not show any significant effect. However, the urine to serum creatinine ratio (U_{Cr}/S_{Cr}) improved significantly when cystone (1000 mg/kg i.p.) was given 1 h before cisplatin. Similar results were obtained in the case of creatinine clearance (Cl_{Cr}). Cisplatin reduced Cl_{Cr} compared to control animals. When cystone was given 1 h before cisplatin the value was found to be similar to control. In the case of urinary excretion of proteins cystone was not able to inhibit the increased excretion of urinary proteins due to cisplatin.

3.5. Effect on WBC

Cisplatin caused significant reduction in the WBC count. However, cystone did not show any protection against the reduction of WBC.

4. Discussion

Although cisplatin is an important anticancer agent, its use is limited because of its renal toxicity. The present study showed that the polyherbal ayurvedic formulation, cystone (1000 mg/kg i.p.) offered significant protection against cisplatin renal toxicity in rats. Reactive oxygen species are

Table 4
Effect of cisplatin and cystone on renal functions

Treatment ^a	U_{Vol} (ml/h per 100 g b.w.)	U_{Cr}/P_{Cr}	Cl_{Cr} (ml/h per 100 g b.w.)	Uprotein (mg/24 h)
Control	1.30 ± 0.15	17.84 ± 3.8	16.2 ± 1.7	5.7 ± 0.8
Cisplatin, 3 mg/kg	0.95 ± 0.15	9.1 ± 0.7*	8.8 ± 1.3*	16.7 ± 2.8*
Cisplatin, 3 mg/kg plus cystone, 1000 mg/kg	1.10 ± 0.10	14.3 ± 2.0 #	15.2 ± 2.5 #	12.8 ± 2.4*

^a Cisplatin was given i.p. and cystone was given 1 h before cisplatin. On day 5, urine and serum were collected to calculate urinary volume (U_{Vol}), urine to serum creatinine ratio (U_{Cr}/S_{Cr}), creatinine clearance (Cl_{Cr}) and urinary excretion of protein ($U_{Protein}$) in rats.

* $P < 0.05$ compared to control.

$P < 0.05$ compared to cisplatin treated.

known to play an important role in the renal toxicity of cisplatin (Inselmann et al., 1995). Our studies also showed that the lipid peroxidation increased significantly when renal cortical slices were incubated with cisplatin. Cystone inhibited this elevation in lipid peroxidation suggesting that its antioxidant properties may be contributing to its protective effect. Many antioxidants have shown such protection (Bull et al., 1988; Baldew et al., 1989). Cystone at 1000 mg/kg protected rats when given i.p. 1 h before cisplatin, as shown by the improvement in many renal functions and body weight. There was no protection when given orally indicating that the active constituents responsible for protective activity may be undergoing metabolism in the oral route. Protective action of cystone was also observed in mice when given i.p. (data not shown). Lower doses than 1000 mg/kg or multiple doses (0.3 or 0.5 g/kg for 3 days, 0.1 g/kg for 5 days) did not improve the results (data not shown).

In conclusion, cystone, a polyherbal ayurvedic preparation used clinically for many urinary complications, has been shown to provide partial but significant protection against renal toxicity induced by the antitumor agent, cisplatin. While the exact mechanism of protection cannot be determined, the ability of cystone to inhibit lipid peroxidation needs further investigation.

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References

- Baldew, G.S., van den Hamer, C.J.A., Los, G., Vermuelen, N.P.E., de Goeij, J.J.M., Mc Vie, J.G., 1989. Selenium induced protection against cis-diaminodichloroplatinum nephrotoxicity in mice and rats. *Cancer Research* 49, 3020–3023.
- Bull, J.M.C., Strelbel, F.R., Sunderland, B.A., Bulger, R.E., Edwards, M., Siddik, Z.H., Newman, R.A., 1988. *O*-(β -hydroxyethyl)-rutoside mediated protection of renal injury associated with cis-diaminodichloroplatinum/hyperthermia treatment. *Cancer Research* 48, 2239–2244.
- Garg, S.K., Singh, R.C., 1985. Role of cystone in burning micturition. *Probe* 2, 119–121.
- Godkar, P.B., 1994. Textbook of Medical Laboratory Technology. Bhalani Publishing House, Bombay.
- Goldstein, R.S., Mayor, G.H., 1983. The nephrotoxicity of cisplatin. *Life Sciences* 32, 685–690.
- Inselmann, G., Blohmer, A., Kottny, W., Nellessen, U., Heidemann, H.T., 1995. Modification of cisplatin-induced renal *p*-aminohippurate uptake alteration and lipid peroxidation by thiols, *Ginkgo biloba* extract, desferroxamine and torbafylline. *Nephron* 70, 425–429.
- Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin-Phenol reagent. *Journal of Biological Chemistry* 193, 265–275.
- Madias, N.E., Harrington, J.T., 1978. Platinum nephrotoxicity. *American Journal of Medicine* 65, 307–314.
- Misgar, M.S., 1982. Controlled trial in 100 cases with nephro-urethrolithiasis with cystone, an indigenous drug and other advanced methods. *Current Medical Practices* 5, 26–29.
- Nadkarni, A.K., 1992. Dr K.M. Nadkarni's Indian Materia Medica, vols. I–II. Popular Prakashan, Bombay.
- Rai, S.C., 1960. Clinical trials with cystone in urolithiasis. *Current Medical Practices* 9, 484–487.
- Rajakumar, D.V., Rao, M.N.A., 1993. Dehydrozingerone and isoeugenol as inhibitors of lipid peroxidation and as free

radical scavengers. *Biochemical Pharmacology* 46, 2067–2072.

Rozeneweig, M., van Hoff, D.D., Slavil, M., Muggia, F.M., 1977. cis-Diamine dichloroplatinum, a new anticancer agent. *Annals of Internal Medicine* 86, 803–809.

Sengupta, S., 1987. Cystone in urinary tract complaints during pregnancy. *Medicine and Surgery* 27, 7–11.

Sharma, B.M., Panagariya, A., Jain, K., 1983. Clinical trial of cystone in various renal disorders. *Probe* 2, 113–114.

Yamasaki, F., Ishibashi, M., Nakakuki, M., Watanabe, M., Shiukawa, T., Mizota, M., 1996. Protective action of ulinastatin against cisplatin nephrotoxicity in mice and its effect on the lysosomal fragility. *Nephron* 74, 158–167.