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Nephroprotective Effect of Watery Extract of *Alternanthera pungens* (Myae-khat-kyet-mauk) in Albino Rats Using Cisplatin-induced Acute Renal Failure

Khin Phyu Phyu[1], Lei Lei Win[1], Khin Mi Mi Lay[1], Kyawt Kyawt Khaing[1], Kyi San[1], Aung Thuya[1], Win Khaing[2] & Kyaw Zin Thant[1]

[1]Department of Medical Research (Upper Myanmar)
[2]Department of Preventive and Social Medicine
University of Medicine (Mandalay)

The present study was conducted to investigate the possible potential nephroprotective activity of watery extract of whole plant of *Alternanthera pungens* in cisplatin-induced nephrotoxic rats. Nephrotoxicity was induced in Wistar strain albino rats by single intraperitoneal (i.p) administration of cisplatin 5 mg/kg (n=6). Effect of concurrent administration of *Alternanthera pungens* watery extract at the doses of 800 mg/kg, 1,600 mg/kg and 3,200 mg/kg body weight (n=6 in each group), given by oral route was determined using serum creatinine, serum urea and change in body weight as indicators of kidney damage. Cystone, polyherbal renal protective drug, was used as standard drug for nephroprotective activity. At the dosages of 1,600 mg/kg and 3,200 mg/kg body weight of the watery extract of *Alternanthera pungens* showed significant decrease in elevated serum urea and creatinine ($p<0.001$) (one-way ANOVA, followed by Dunnetts test) when compared to that of toxic group. Both groups showed significant protective activity in cisplatin-induced nephrotoxic rats. At the dose of 1,600 mg/kg watery extract and cystone standard were found to normalize the serum urea and creatinine levels when compared to normal control. It was observed that the watery extract of *Alternanthera pungens* significantly protected the kidneys from injury. The current study revealed that the watery extract of *Alternanthera pungens* had promising nephroprotective activity and was comparable to cystone in the animal model.

INTRODUCTION

Nephrotoxicity is of critical concern during the early stages of drug development when selecting new drug candidates. Because of its unique metabolism, the kidney is an important target of the toxic effect of drugs, xenobiotic and oxidative stress.[1] Cisplatin (Cis-diaminedichloro platinum II, CDDP) is a potent antitumor agent, extensively used for treatment of several cancers like testicular and lungs cancer. Unfortunately, the gracious drug cisplatin is conjoined with a brutal side effect since it induces nephrotoxicity.[2] The injection of cisplatin produced proximal and distal tubular necrosis, mainly in the corticomedullary region and intratubular casts in the outer stripe of the outer medulla. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules.[3]

Cisplatin decreases antioxidants and antioxidant enzymes[4, 5] leading to enhanced generation of reactive oxygen metabolites and lipid peroxidation. A large number of herbs have traditionally been used to treat drug or toxin induced renal diseases.[6] It is reported that many Indian medicinal plants show beneficial effects against renal injury.[7] *Alternanthera pungens* is used traditionally against dysentery, venereal diseases, cholera, and many parasitic diseases and also this plant showed antioxidant property.[8] It is

interesting and important to verify whether their traditional uses are supported by scientific data or merely based on folklore.[9]

The present study was conducted to investigate the possible potential nephroprotective activity of watery extract of whole plant of *Alternanthera pungens* in cisplatin-induced nephrotoxic rats.

MATERIALS AND METHODS

Drugs

Cisplatin (Platinex) (Khamdelwal Laboratories Pvt. Ltd. India) was used to induce nephrotoxicity. Cystone syrup (Himalaya Drug Company, Bangalore, India) was used as standard positive control drug.

Chemicals

For the evaluation of nephroprotective activity, serum urea and creatinine were estimated by the use of commercial kits from Hospitex Diagnostics (Italy). For testing antioxidant activity, 1, 1-diphenyl -2-picryl-hydrazyl (DPPH), ascorbic acid (Vitamin C) and distilled water were used.

Analytical instruments

- Balance-AW 220, Shimadzu, Japan
- UV Spectrophotometer, 1601(Shimadzu)
- Vortex mixer
- Biochemical analyzer & Standard accessories: Screen Master Touch, Hospitex Diagnostics (Italy)

Plant material

Whole plants of *Alternanthera pungens* were collected from Amarapura, Mandalay Region in March, 2012. The plants recommended by local traditional practitioners to treat renal disease were taxonomically authenticated at the Department of Botany, University of Mandalay.

The collected plants were carefully washed and dried under continuous ventilation in the laboratory until total dryness was obtained. The samples were then powdered with a grinder and stored at room temperature until use.

Experimental animals

Forty healthy albino mice (ICR strain) of both sexes with average weight of 25-30 gm were used for acute toxicity test. Thirty-six healthy albino rats (Wistar strain) of both sexes with an average weight of 180-250 gm were used for testing nephroprotective activity. The animals had free access to standard pellet diet and water *ad libitum.*

Preparation of the extract

One-hundred grams of dried coarse powder were extracted with 1,500 ml of distilled water in a boiling water bath for 6 hours at 60°C.[10] Then, the extract was concentrated in vacuum under reduced pressure and evaporated at 50°C until solid residue was obtained.

Preliminary phytochemical analysis

The watery extract of the whole plant of *Alternanthera pungens* was subjected to preliminary phytochemical analysis for detecting various phytoconstituents.[11]

1,1-Diphenyl-2-picryl-hydrazyl(DPPH) radical scavenging assay

Determination of radical scavenging activity of DPPH method is based upon the change on absorbance of watery extract solution in various concentrations. The principle is that, in the presence of a stable free radical (DPPH), antioxidant donates a hydrogen atom to quench the stable free radical. Due to the ethanolic component, DPPH solution appears violet and at 517 nm, the color of which changes upon neutralization by free radical to pale yellow.[12]

Acute oral toxicity study

Acute oral toxicity study was done to determine the median lethal dose (LD_{50}) according to the method of Litchfield and Wilcoxon.[13] Thirty albino mice in three groups (10 mice/ group) were orally given watery extract of *Alternanthera pungens*, at the doses of 1,600 mg/kg body weight, 3,200 mg/kg body weight and 6,400 mg/kg body weight, respectively. The control group (10 mice/ group) received 10 ml/kg body

weight of distilled water. All the animals were kept under observation for screening of toxic symptoms for 2 weeks.

Nephroprotective activity

The rats were divided into six groups, each containing six animals for this study.

Group I

Animals received distilled water for 10 days as normal control.

Group II

Animals received distilled water from day 1 to day 10 and received cisplatin, 5 mg/kg, i.p., single dose on day 11 as toxic control.

Group III

Animals received cystone syrup, 5 ml/kg body weight once daily (o.d) orally from day 1 to day 10 and cisplatin, 5 mg/kg, i.p., single dose on day 11 as standard.

Group IV, V & VI

Animals received watery extract of *Alternanthera pungens* 800 mg/kg body weight, 1,600 mg/ kg body weight, and 3,200 mg/kg body weight orally o.d, respectively, from day 1 to day 10 and cisplatin, 5 mg/kg, i.p., single dose on day 11 as the test groups for nephroprotective activity.

All experimental groups had free access to standard pellet diet and water *ad libitum* from day 1 to day 15. On day 16, animals were anaesthetized by chloroform and sacrificed. Blood samples were collected by cardiac puncture for its biochemical parameters. Kidneys were dissected out immediately and transferred into 10% formalin for further histopathological studies.[14]

Parameters assessed for renal toxicity

Body weight

The body weight (in grams) of the animals were recorded on the first and last day of experiment and the percent reduction was calculated.

Serum urea and creatinine

Urea and creatinine levels in serum were determined by enzymatic method using Hospitex Diagnostics kits (Italy).

Histopathological studies

Formalin preserved samples of kidneys from various experimental groups were studied for both gross and histopathological changes during experiment. Then, weights of the kidneys of all groups were noted immediately after blood collection. Sections of kidneys, stained with haematoxylin and eosin, were observed under standard microtechnique.

Statistical analysis

Data were statistically analyzed by Student 't' test and all values were expressed as Mean±SE. Data were also analyzed by one-way ANOVA, followed by Dunnetts comparison and p values <0.001 were considered as significant.

RESULTS

Botanical investigation of "Myae-khat-kyet–mauk" confirmed it as *Alternanthera pungens.* Yield percentage of the watery extract of the whole plant of *Alternanthera pungens* was 17.6 (w/w) and the results of phytochemical tests are shown in Table 1.

Table 1. Phytochemical screening of watery extract of *Alternanthera pungens*

Compound	Reagent	Test	Inference
Alkaloids	Dragendroff's	Dragendroff's	-
Flavonoids	Dil: HCl, Zn or Mg	Flavonoids	-
Glycosides	10% Lead acetate	Lead acetate	+
Steroid	Conc: H_2SO_4, $CHCl_3$, acetic anhydride	Liebermann Burchard's	+
Phenols	10% $FeCl_3$	Ferric chloride	+
Carbohydrate	∞Naphthol, Conc:H_2SO_4	Molisch	+
Saponins	Water	Foam	+
Tannins	1%$FeCl_3$, dil: H_2SO_4	Ferric chloride	+
Tri-terpene	Conc: H_2SO_4, $CHCl_3$, acetic anhydride	Liebermann Burchard's	+
Protein	10% NaOH, 3% $CuSO_4$	Protein	+
∞amino acid	Ninhydrin	Ninhydrin	-
Reducing sugar	Benedict's solution	Benedict's	+
Resin	Acetic anhydride, H_2SO_4	Resin	-

(+)=presence, (-)=absence

The free radical scavenging activity expressed as 50% Inhibitory Concentration (IC_{50})

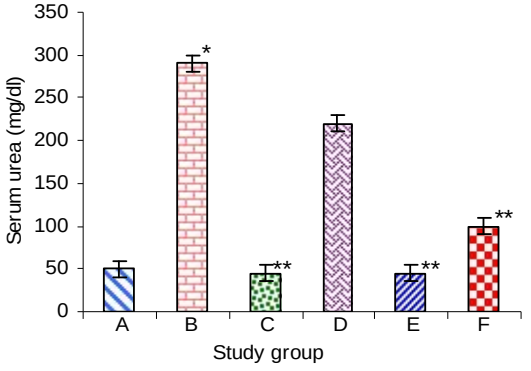
of the watery extract *Alternanthera pungens* was 0.09 µg/ml and that of ascorbic acid was 0.49 µg/ml. Acute toxicity test did not show mortality of animals in any doses tested. Therefore, it was concluded that median lethal dose (LD_{50}), when administered orally, was supposed to be more than 6,400 mg/kg body weight. The percent reduction of body weight, kidney weight, serum creatinine and urea data are shown in Table 2.

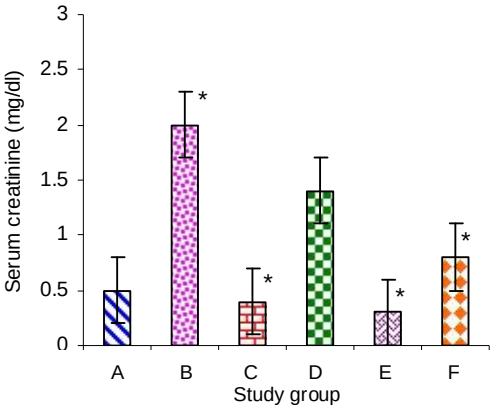
Table 2. Nephroprotective effect of watery extract of *Alternanthera pungens* on different physical and biochemical parameters

Gps	Treatment	Parameters			
		Physical		Biochemical	
		Reduction of B.W (%)	Kidney weight (g)	Serum urea (mg/dl)	Serum creatinine (mg/dl)
I	Normal control	7.2	2.25 ±0.13	52.16 ±2.19	0.54 ±0.02
II	Cisplatin 11th day (toxic)	23.4	1.35 ±0.06*	287.87 ±32.39*	2.02 ±0.37*
III	Cystone+cisplatin (standard)	1.2	1.93 ±0.19	45.4 ±2.36**	0.48 ±0.03**
IV	Watery extract 800 mg/kg B.W+ cisplatin	11.1	1.53 ±0.19	219.75 ±18.87	1.29 ±0.16
V	Watery extract 1,600 mg/kg B.W+ cisplatin	3.1	1.82 ±0.12	44.62 ±1.66**	0.47 ±0.01**
VI	Watery extract 3,200 mg/kg B.W+ cisplatin	14.2	1.42 ±0.04*	107.51 ±13.38**	0.84 ±0.6**

Gps=Groups, B.W=Body weight, *=Compared to control, **=Compared to cisplatin

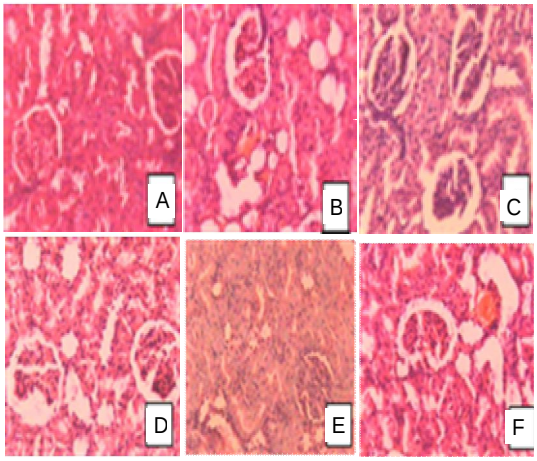
The effect of cisplatin and watery extract of *Alternanthera pungens* on serum urea and creatinine are shown in Fig. 1 a & b. Data were statistically analyzed by Student 't' test and all values were expressed as Mean±SEM for 6 animals in each group.





A = Normal control
B = Cisplatin
C = Cystone+Cisplatin
D = Watery extract 800 mg/kg B.W+Cisplatin
E = Watery extract 1600 mg/kg B.W+Cisplatin
F = Watery extract 3200 mg/kg B.W+Cisplatin

Fig. 1 a & b. Effect of cisplatin and watery extract of *Alternanthera pungens* on serum urea and creatinine (All values are expressed as mean±SEM (n=6); *p=<0.001 compared to control, **p=<0.001 compared to cisplatin.)



A =Normal control kidney showing normal glomeruli and tubules
B =Toxic group (11th day, cisplatin) kidney showing congestion of glomeruli capillaries and stroma congestion
C =Standard group (cystone+cisplatin) kidney showing normal glomeruli and tubules
D =Watery extract (800 mg/kg B.W+cisplatin) kidney showing normal glomeruli and tubules
E =Watery extract (1,600 mg/kg B.W+cisplatin) kidney showing normal glomeruli and tubules
F =Watery extract (3,200 mg/kg B.W+cisplatin) kidney showing mild congestion of stroma of tubules

Fig. 2. Histopathologic evaluation of rat kidneys

Data were also analyzed by one-way ANOVA, followed by Dunnetts comparison and p values <0.001 were considered as significant: ∗p<0.001 compared to control, **p<0.001 compared to cisplatin. Kidney weights were significantly decreased (p<0.001) in toxic and 3,200 mg/kg body weight dose groups when compared with control group. Glomerular congestion of capillaries was found in some rats of group II. Mild congestion of tubular stroma was found in some rats of group VI. The photomicrograph of tissue sections are presented in Fig. 2.

DISCUSSION

Cisplatin is a potent anticancer agent used in solid tumours of testes, ovary, breast, lungs, bladder etc. However, its clinical use is limited by its renal toxicity.[14] Cisplatin accumulates in the renal tubular cells approximately 5 times its extracellular concentration, causing the impairment of kidney. Therefore, it is recognized as the main side effect and the dose limiting factor associated with its use. The mechanism of cisplatin-induced nephrotoxicity is complex and involves oxidative stress, apoptosis, inflammation and fibrogenesis.[1]

Nephrotoxicity is an increasingly common and potentially catastrophic complication in hospitalized patients. Early observational studies from the 1980's and 1990's established the general epidemiologic features of acute kidney injury. Kidney is mainly affected by many chemicals and drugs. Drug-associated nephrotoxicity accounts for 18-27% of all acute kidney injury cases in US hospitals. There is no specific treatment to reverse the nephrotoxicity, but it may be reduced with the symptomatic treatment.[1]

Present findings of significant increase in serum urea and creatinine levels (p<0.001) and the decrease in body weight in toxic group when compared to the normal control, indicated the induction of nephrotoxicity with cisplatin.

Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules. Because this renal damage occurs in the first hour after administration, it is important that the protective agent needs to be present in sufficient concentrations in renal tissues before the damage occurs. This is the rationale behind the prophylactic treatment.[4]

In this study, prophylactic activity of watery extract of *Alternanthera pungens* was tested with three different doses. At the dosages of 1,600 mg/kg and 3,200 mg/kg body weight of the watery extract of *Alternanthera pungens*, significant decreases were observed in cisplatin-induced elevated serum urea and creatinine (p<0.001) when compared to those of toxic group. Both groups showed significant protective activity in cisplatin-induced nephrotoxic rats.

At the dose of 800 mg/kg body weight of the watery extract of *Alternanthera pungens,* no significant decrease in elevated serum urea and creatinine was observed when compared to that of toxic group. Cystone was reported to protect against cisplatin-induced toxicity and protection may be mediated through its ability to inhibit lipid peroxidation.[14] In this study, cystone (standard) showed significant decrease in the elevated serum urea and creatinine (p<0.001) when compared to that of toxic group.

The result of 1,600 mg/kg watery extract of *Alternanthera pungens* was found to decrease in serum markers when compared with cystone (standard) but the difference was not statistically significant. Both groups of 1,600 mg/kg watery extract and cystone standard were found to normalize the serum urea and creatinine levels when compared to normal control. The results of present study revealed the promising significant prophylactic activity by 1,600 mg/kg body weight dose of watery extract of *Alternanthera pungens*.

The biochemical results were also supported by histopathological features of the kidney. Normal glomeruli and tubules were observed

in 800 mg/kg, 1,600 mg/kg body weight and control groups, respectively. Only mild congestion of tubular stroma was observed at 3,200 mg/kg dose of watery extract of *Alternanthera pungens* in some rats and also mild congestion of glomeruli capillaries were observed in cisplatin treated group.

In conclusion, our study showed that the whole plant of *Alternanthera pungens* possess marked nephroprotective activity at the dose of 1,600 mg/kg body weight and thus, it played a promising role in the treatment of acute renal injury. The exact mechanism of protection cannot be determined by the present study. However, the ability of the constituents of phenol and tannin in the watery extract to scavenge free radicals may possibly involve in the protection mechanism. However, caution should be exercised in its use especially at high dose of the extract, 3,200 mg/kg body weight due to the possibility of toxicity. Further study for isolation of active components of *Alternanthera pungens* and evaluation of its nephroprotective activity in chronic renal failure model should be performed.

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