NEPHRO-PROTECTIVE ACTIVITY OF AQUEOUS EXTRACT OF *AERVA JAVANICA* ROOTS IN CISPLATIN INDUCED RENAL TOXICITY IN RATS

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Summary

Aerva javanica (Amaranthaceae) is a tall and woolly under shrub found plentiful in rainy season in crevices of rocks in Gujarat state. It is possessing medicinal properties like antirheumatic, antiurolithiatic, anthelmintic, demulcent, swellings and acne disorders etc. In spite of manifold uses the plant in kidney disorders it was not evaluated for nephro-protective activity. Hence the present study provides the scientific evaluation of nephro-protective activity of aqueous extracts of *Aerva javanica* roots. Six groups of six wistar albino rats were used for cisplatin induced nephrotoxicity. In this model group-I was administered with acacia (2%) (control-1), group-II was treated with aqueous extract (control-2), group-III with cisplatin (5mg/kg), group-IV cisplatin (5mg/kg) with equivalent volume of gum acacia solution, group-V cisplatin (5mg/kg) + 200mg/kg aq. extract, group-VI cisplatin (5mg/kg) + 400mg/kg aqueous extract was used. Various parameters like body weight, blood urea, serum creatinine, serum protein, total protein, serum albumin, urine volume and pH, tissue protein, GSH and TBARS level were compared with controls on 16th day after treatment. From our study it was observed that cisplatin injury was evidenced by the elevated biochemical markers [blood urea, serum creatinine, total protein and serum albumin, urine volume, urine P^H] and histopathological features of acute tubular necrosis. The aqueous extract at the dose level of 400 mg/kg body weight was found to normalize the elevated biochemical markers and bring about a marked recovery in kidneys as evidenced microscopically.

Key words: Nephro-protective, *Aerva javanica*, aqueous extract, Cisplatin

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Introduction

A large number of chemicals in common usage nowadays are renal toxins. Administration of such toxins into the body may cause mechanical trauma to the kidneys and selectively interfere with certain functions of the renal tubules. Proximal renal tubular cells are particularly susceptible to acute injury by these substances and the exposure may be followed by acute tubular necrosis[1,2,3]. Certain food dyes are known to cause renal toxicity[4]. It has also been found that chronic exposure to hydrocarbon solvents used in industry may induce a form of proliferative glomerulonephritis mediated by antiglomerular basement membrane antibody[5].

Acute Renal Failure (ARF) and Chronic Renal Failure (CRF) are two major complications in relation to the kidney. ARF is reversible loss of kidney function where as CRF is irreversible loss of kidney function. Various causes have been attributed to renal failure like glomerular nephritis, gout, hypertension, diabetes mellitus, antineoplastic agents like cyclophosphamide, vincristine, cisplatin etc[6].

Ancient literature has prescribed various herbs for the cure of kidney disease. The term "Pasanabheda" has been cited in the literature to identify a group of plants, which have been extensively, used in the indigenous system of medicine to dissolve urinary calculi and stones.

Aerva javanica is reported as anthelmintic, diuretic, demulcent[7,8,9]. It is used for the treatment of headache[9]. The decoction of the plant is administered to remove swellings[8,10], applied to acne like conditions of the face[11].

Materials and methods

Collection: Fresh roots of *Aerva javanica* were collected from Bhavnagar District, Gujarat. The plant was authenticated by botanist. The herbarium specimen bearing voucher No.: PP.553 has been deposited in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India. Shade dried roots (500g) were coarsely powdered and macerated with 1% chloroform-water for seven days. The extract was filtered and concentrated *in vacuo* to syrupy consistency.

Phytochemical analysis: Aqueous extract of root was qualitatively tested for identification of various phytoconstituents according to standard methods[12].

Animals: Healthy adult male albino rats of Wistar strain weighing between 150 - 250g aged 60 - 90 days were used for the study. The rats were housed two in a cage, maintained in a temperature regulated and humidity controlled environment. The rats were fed with standard food pellets and water. Study was conducted after obtaining ethical committee clearance from the Institutional Animal Ethics Committee of K.M.C., Manipal No. IAEC/KMC/06/2006-2007.

Acute toxicity study: Oral acute toxicity studies were carried out with rats, weighing 150-250 g, with 2 rats per dose group. The extracts were administered in a staircase method (Ghosh, 1984). The rats were fed with aqueous (AQ) root extract of *Aerva javanica* suspended in 2% gum acacia at dose 2000-mg/kg body weight. The animals were observed continuously for 2 hours for the gross behavioral changes and then intermittently once in every 2 hours and finally at the end of 24 and 72 hours to note for any signs of toxicity including death.

Nephro-protective activity:

Cisplatin induced renal toxicity[13]: Six groups of six rats each were used for aqueous extracts. Further procedure was followed according to explained in table 1.

Table: 1. Experimental protocol for aqueous extract of *Aerva javanica* in cisplatin model of nephrotoxicity.

G.No. (n=6/ group)	Drug treatment	Route and dose	Duration (in days)	Days of withdrawal of blood and kidney	Purpose
I	Gum acacia 2%)	5 ml/kg p.o.	1^{st} – 15^{th}	16^{th}	Control
II	AQ extract	400 mg/kg p.o.	1^{st} – 10^{th}	11^{th}	Control
III	Cisplatin	5 mg/kg i.p. (single dose)	1^{st}	6^{th}	Induce kidney damage
IV	Cisplatin + Gum acacia	5 mg/kg i.p. (single dose) equivalent volume	1^{st} 6^{th} –15^{th}	16^{th}	Induce kidney damage and to check regeneration
V	Cisplatin + AQ. Extract	5 mg/kg i.p. (single dose) 200 mg/kg p.o.	1^{st} 6^{th} – 15^{th}	16^{th}	Curative effect
VI	Cisplatin + AQ. Extract	5 mg /kg i.p. (single dose) 400 mg/kg p.o.	1^{st} 6^{th} – 15^{th}	16^{th}	Curative effect

The dose of the extract was calculated as $1/10^{th}$ of the maximum tolerated dose (2000 mg/kg body weight).
i.p.: intra peritoneal, p.o.: per oral

The blood withdrawn from each group was used to estimate serum creatinine, blood urea, total protein and albumin. Urine was collected using metabolic cage from each group used to estimate urine volume, urine PH, urine calcium level and urine uric acid. Kidneys were removed and homogenated to carry out the estimation of tissue protein, Glutathion (GSH), and lipid peroxidation

(TBARS). Kidneys were given for histopathological examination to determine the extent of tissue damage/healing.

Statistical analysis

The data was analyzed using One-Way ANOVA followed by Post Hoc Sheffe's Test using SPSS computer software version 7.5. Level of Significance was fixed at 0.05.

Results

Acute toxicity studies: Administration of aqueous extracts of root of *Aerva javanica* orally up to 2000-mg/kg body weight dose was given to rats. After 72 hours of observation, any side effect was not observed in rats. There was no case of mortality. Up to 2000 mg/kg dose was found to be safe.

Cisplatin induced renal toxicity: Significant reduction was observed in body weight, total protein, serum albumin, protein and glutathione (GSH) of rats in groups III and IV when compared with group I, where as in curative groups V and VI showed the recovery from the decreased level. Blood urea, serum creatinine and lipid paroxidase enzyme (TBARS) were significantly increased in rats of groups III and IV when compared to group I while, in curative groups V and VI showed the normal level from raised level. (Table-2.1, 2.2). Histopathological changes were observed in before and after treatment of aqueous extract in Cisplatin induced renal toxicity in rats.(Table-3)

Table: 2.1 Effect of aqueous extract of *Aerva javanica* in Cisplatin induced renal damage.

Groups	% Change in body weight	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Blood protein (gm/dL)	Albumin (gm/dL)
Control	2.23 ± 0.95	38.5 ± 4.0	0.70 ± 0.10	6.55 ± 0.05	4.00 ± 0.10
AQ. 400mg/kg B.W	14.04 ± 1.86	38.5 ± 0.05	0.60 ± 0.00	6.85 ± 0.35	4.05 ± 0.15
Cisplatin 6th day	-4.89 ± 1.43	325.5 ± 31.59	4.70 ± 0.60	5.80 ± 0.30	3.15 ± 0.05
Cisplatin 16th day	-7.07 ± 0.39	335 ± 1.0	5.20 ± 0.10	5.35 ± 0.30	2.85 ± 0.05
AQ. 200mg/kg B.W	3.24 ± 0.76	160.0 ± 18.05	2.55 ± 0.15	6.70 ± 0.10	3.45 ± 0.45
AQ. 400 mg/kg B.W	6.50 ± 1.25	53.14 ± 14.04	0.80 ± 0.10	6.85 ± 0.05	3.70 ± 0.30

Values are Mean ± S. E.

Table: 2.2 Effect of aqueous extract of *Aerva javanica* in Cisplatin induced renal damage.

Groups	Urine volume (ml)	Urine pH	Protein mg/ml	GSH µmol/mg Protein	TBARS nM/mg Protein
Control	5.5 ± 0.5	9.0 ± 0.0	194.03 ± 3.33 E-06	12.23 ± 0.023	145.36 ± 0.336
AQ. 400mg/kg B.W	4.5 ± 0.5	9.0 ± 0.0	195.75 ± 0.001	13.46 ± 0.023	145.06 ± 0.333
Cisplatin 6th day	15.5 ± 0.5	7.5 ± 0.5	100.67 ± 6.66 E-05	6.41 ± 0.004	384.09 ± 0.064
Cisplatin 16th day	19 ± 1.0	6.5 ± 0.5	88.32 ± 5.77 E-05	6.39 ± 0.005	463.05 ± 0.739
AQ. 200mg/kg B.W	9.0 ± 1.0	9.0 ± 0.0	129.35 ± 8.81 E-05	7.36 ± 0.003	283.46 ± 0.500
AQ. 400 mg/kg B.W	6.0 ± 0.0	9.0 ± 0.0	153.04 ± 3.33 E-06	7.70 ± 0.002	235.81 ± 0.426

Values are Mean ± S. E.

Histopathology

Table: 3. Effect of aqueous extract of *Aerva javanica* on histopathological features as seen in the kidney in the cisplatin model.

Histopathological changes	GP-I	GP-II	GP-III	GP-IV	GP-V	GP-VI
Glomerular congestion	-	-	++	+++	+	-
Tubular casts	-	-	++	+++	-	-
Peritubular congestion	-	-	+	+++	+	-
Epithelial desquamation	-	-	++	++	-	-
Blood vessel congestion	-	-	++	+++	+	-
Inflammatory cells	-	-	-	-	-	-

- absent, + present, ++ more, +++ most

Discussion

From our study it was observed that cisplatin induced renal injury was evidenced by the elevated biochemical markers [blood urea, serum creatinine, total protein and serum albumin, urine volume, urine pH] and by the histopathological features of acute tubular necrosis. The aqueous extract of *Aerva javanica* at a dose level of 400 mg/kg body weight was found to normalize the changed blood urea, serum creatinine, total protein and serum albumin, urine volume, urine pH and bring about a marked recovery in kidneys as evidenced microscopically.

Previous reports suggest that cisplatin induced nephrotoxicity is by initiation of lipid peroxidation and depletion of cellular thiols[15]. Cisplatin inhibits the activity of antioxidant enzymes (Glutathoine and lipid peroxidase) in rat kidneys[16] suggesting that cisplatin cytotoxicity results from the generation of reactive oxygen species (ROS). The results obtained in this study correlate with previous reports that lipid peroxidation contributes to cisplatin induced nephrotoxicity.

In the present work aqueous extracts of *Aerva javanica*, was found to increase the GSH and tissue protein level and to decrease the concentration of TBARS in kidney. Hence the possible mechanism of nephroprotection by this plant may be attributed to its antioxidant & free radical scavenging properties.

Conclusion

To conclude, our studies have shown that the root of plant *Aerva javanica* possesses marked nephroprotective activity with minimal toxicity and could have a promising role in the treatment of acute renal injury induced by nephrotoxins, especially cisplatin. Further work envisages evaluating its nephroprotective activity in chronic renal failure models.

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