ABSTRACT

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Aerva lanata is a medicinal herb that has been used as nephroprotective agent in Chinese medicine. In this study, the protective effect of Aerva lanata against cisplatin-induced nephrotoxicity in rats was evaluated. Nephrotoxicity was induced in male albino rats by intra peritoneal administration of cisplatin 150 mg/kg/day for 7 days. Effect of concurrent administration of ethanol extract of Aerva lanata at a dose of 150 mg/kg/day given by oral route was determined using serum urea, creatinine, total protein, albumin, sodium, potassium, MDA, GSH. Result of the present study suggest that the extract possesses significant nephroprotective activity.

KEYWORDS: Aerva lanata, Cisplatin, Electrolytes, Nephroprotection.

INTRODUCTION

The kidney consists of over 20 cell types that differ in function, metabolism and ultra structure. The entire kidney is highly compartmentalized, due to the structural organization,[1] as well as the functional[2] and biochemical segmentation of the nephron. Some chemicals may thus be selectively concentrated in discrete areas of the kidney.[3] They induce very selective lesions in a specific cell type, Cisplatin is a major antineoplastic drug used for the treatment of solid tumors. Its chief dose limiting side effect is nephrotoxicity and may provoke a cascade of secondary degenerative events. These events may extend progressively to more of the renal cell types that have a greater or lesser capacity for repair. This chain reaction may cause renal failure when there is a decreased renal functional reserve.[4] Cisplatin induces glucose-6-phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production. These
free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, which lead to enzymatic inactivation. Free radicals can also cause mitochondrial dysfunction.

Cisplatin nephrotoxicity primarily causes tubulo interstitial lesions. The site of injury involves either the distal tubule and collecting ducts or the proximal and distal tubules.[5]

There is a continuous search for agents which provide nephro protection against the renal impairment caused by drugs like Cisplatin for which allopathy offers no remedial measures. Thus, it is imperative that mankind turns towards alternative systems of medicine for solace.

*Aerva lanata* is a much-branched herb, with the base hard as wood and the branches erect or creeping to the ground. The plant is astringent, bitter, cooling emollient, vermifuge, suppurative, diuretic and lithontriptic. It is useful to treat boils, cephalopathy, cough, strangury, diabetes and lithiasis useful in catarrh of bladder, tonic, flowers used for removal of kidney stone.[6]

Hence in the present study attempts are made to evaluate the nephroprotective effect of methanolic extract of *Aerva lanata* leaves in cisplatin induced nephro toxic rats.

**MATERIALS AND METHODS**

**Animals**

Male albino rats of Wistar strain approximately weighing 150-125g were used in this study. They were healthy animals bought from animal house, Annamalai University, Chidambaram. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fiber (4.02%), Ash (8.02%) and sand silica (1.02%).

**Plant Material and Preparation of Extract**

The leaves of *Aerva lanata* were collected from Sengipatti, Thanjavur District. The collected leaves were cut into small pieces and shade dried at room temperature. The leaves were soaked with methanol (50%) for 48 hours. A semi solid extract was obtained after complete
elimination of alcohol under reduced pressure. The extract contained both polar and non-polar phytocomponents of the plant material used. The extract was stored in refrigerator until used. It was dissolved in distilled water just before oral administration.

Experimental Design

Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows.

**Group 1:** Normal control rats, fed with standard diet and received intra peritonial injection of isotonic saline for 7 consecutive days.

**Group 2:** Rats received i.p. injection of cisplatin (5 mg/kg body weight) for 7 consecutive days.

**Group 3:** Rats were treated with *Aerva lanata* (through intragastric tube) at the dose of 500 mg/kg body weight for every day in addition to injection of Cisplatin for 7 consecutive days.

Collection of samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected without EDTA as anticoagulant, Serum was separated by centrifugation. Kidney was excised immediately and immersed in physiological saline. The 10% homogenate was prepared by using phosphate buffer (pH 7.4).

Biochemical estimations

Malondialdehyde was estimated by Thiobarbituric acid assay method. Reduced glutathione was estimated by the method of Moron *et al* (1979). Serum urea was estimated by the method of Berthelot (1959). Serum creatinine was estimated by alkaline picrate method. Serum sodium was estimated by the method of Maruna & Trindors (1958). Serum potassium was estimated by turbidometric method. Protein was estimated by the method of Lowry *et al* (1951). Albumin was estimated by the method of Rodkey (1965).

Statistical analysis

The results were presented as mean ± SD. Data was statistically analyzed using student “t” test. P values set as lower than 0.05 was considered as statistically significant.
RESULTS AND DISCUSSION

Cisplatin is an effective chemotherapeutic agent that is widely used for the treatment of malignant tumors including head and neck, ovarian, testicular, lung and breast cancers.[15] Despite the antineoplastic efficacy, the optimal clinical usefulness of cisplatin is usually limited due to its dose-related nephrotoxicity.[16] Acute renal injury can occur after an initial dose of cisplatin with about 20% of patients experiencing various degrees of renal dysfunction.[17] Cisplatin exerts its nephrotoxic effect mainly in the proximal tubular cells where it is preferentially accumulated.[18] The precise mechanisms underlying this toxicity are not fully elucidated. However, oxidative stress with increased generation of reactive oxygen species, and inflammation with increased production of pro-inflammatory cytokines seem to play a crucial role.[19] Several antioxidants and anti-inflammatory agents were proved effective in protecting the kidney against the deleterious effects of cisplatin.[20, 21]

Reactive oxygen species may produce cellular injury and necrosis via, several mechanisms including peroxidation of membrane lipids, protein, denaturation and DNA damage.[22] Ramasamy et al. (1986)[23] have also demonstrated that there is an increase in renal cortical lipid peroxidation in Cisplatin-treated rats. In this context, a marked increase in the concentration of serum and kidney MDA were observed (Table 1) in Cisplatin induced rats when compared to control rats. Administration of Aerva lanata significantly decreased the levels of MDA in Cisplatin-induced rats. This view is supported by previous studies.[24]

Glutathione is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydro peroxides. Glutathione status is a highly sensitive indicator of cell functionality and viability. GSH depletion is linked to a number of disease states including cancer, neuro degenerative diseases, kidney and cardiovascular diseases. Kidneys are exposed to various cytotoxic agents before the elimination of these agents in urine. Thus the GSH concentrations in kidney cells are important.[25] In the present study, declined (Table 1) level of GSH was observed in Cisplatin-induced rats when compared to control rats. The decrease in GSH level represents increased utilization for neutralizing free radicals generated from Cisplatin. Supplementation of Aerva lanata to Cisplatin induced rats, brought back the values to near normal level. The results are compatible with the study of Silan et al. (2007).[26]
Urea is an end product of protein catabolism. It is freely filtered by the glomerulus, passively reabsorbed in both the proximal and distal nephron and excreted in high concentration in urine. The excretion of urea was recognized as an estimate of kidney function. The serum urea level is used as an index of kidney function. Drugs that can increase urea levels include allopurinol, some diuretics, Cisplatin and indomethacin. Nephrotoxicity induced by Cisplatin is a complex phenomenon characterised by an increase in blood urea concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure. In the present study, increased level of urea in Cisplatin intoxicated rats was observed (Table 2). Supplementation of Aerva lanata restored the increased level of urea in Cisplatin-induced rats. This result is supported by earlier studies.

Creatinine is an end product of muscle catabolism, which is removed at a constant rate by the kidneys. The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine. The clinical utility of the serum creatinine concentration centers on its relation to the glomerular filtration rate (GFR). The serum creatinine concentration is the most commonly used index of the kidney function. The level of creatinine in the blood rises if the kidney does not function properly. Cisplatin has been reported to increase creatinine measurements. This result supports our findings (Table IV). Administration of Aerva lanata restored the level of creatinine in Cisplatin treated rats. The present study is consistent with the studies of.

Proteinuria, most often reflecting loss of the normal glomerular impermeability to filtration of plasma proteins, is an early sign of kidney disease. Proteinuria is a hallmark of kidney disease. Thus detection of proteinuria is necessary for the recognition of most kidney diseases. In the present study, plasma albumin and protein were found to be decreased significantly in Cisplatin induced rats when compared to normal rats (Table 3), may be due to the toxicity of free radicals generated from Cisplatin which damaged nephron and thereby loss of protein through urine. Administration of Aerva lanata normalized the level of serum albumin and protein in Cisplatin treated rats.

Electrolyte imbalance can lead to serious consequences as it affects the homeostasis of the body. Homeostasis is the process by which the body cells maintain their internal balance in spite of changes in the external environment. Commonly measured electrolytes are sodium, potassium, calcium, chloride bicarbonate etc., which are good indicators of kidney function. In the present study, Cisplatin treated rats were presented with significantly lower serum
potassium levels and higher sodium levels (Table 4) when compared with normal control rats. This might have been due to the imbalance in the antiport system of sodium and potassium i.e. the increased excretion of potassium promoted the reabsorption of sodium. Administration of *Aerva lanata* restored the normal level of sodium and potassium in Cisplatin treated rats.

The results of the present study prove the efficacy of *Aerva lanata* as a nephro protective agent. Further studies are required to isolate the active principles responsible for nephro protection and to elucidate their mechanism of action at the molecular level.

**Table 1: Effect of *Aerva lanata* leaves extract on MDA and GSH in experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (µmole of MDA formed/dL)</th>
<th>GSH (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>5.573 ± 1.55</td>
<td>12.38 ± 0.67</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>12.71 ± 1.32*</td>
<td>8.74 ± 0.43*</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>9.16 ± 0.74**</td>
<td>11.73 ± 0.61**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats.

*Compared with group I normal rats (p< 0.001)

**Compared with group II control rats (p< 0.001)

**Table 2: Effect of *Aerva lanata* leaves extract on Urea and Creatinine in experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>20.64 ± 1.51</td>
<td>1.07 ± 0.38</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>106.47 ± 7.74*</td>
<td>1.85 ± 0.25*</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>23.73 ± 1.77**</td>
<td>1.12 ± 0.07**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD for six rats.

*Compared with group I normal rats (p< 0.001)

**Compared with group II control rats (p< 0.001)

**Table 3: Effect of *Aerva lanata* leaves extract on total Protein and Albumin in experimental rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Total Protein (gm/dL)</th>
<th>Albumin (gm/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>6.47 ± 0.44</td>
<td>3.17 ± 0.40</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>4.20 ± 0.95*</td>
<td>1.93 ± 0.30*</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>7.72 ± 0.37**</td>
<td>3.13 ± 0.30**</td>
</tr>
</tbody>
</table>

*Compared with group I normal rats (p< 0.001)

**Compared with group II control rats (p< 0.001)
Table 4: Effect of Aerva lanata leaves extract on Sodium and Potassium content in experimental rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Sodium (Mequ/dL)</th>
<th>Potassium (Mequ/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td>140.7 ± 0.67</td>
<td>5.23 ± 0.95</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td>200.01 ± 10.29*</td>
<td>3.23 ± 0.32*</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td>140.5 ± 0.69**</td>
<td>5.23 ± 0.06**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±s SD for six rats.

*Compared with group I normal rats (p< 0.001)

**Compared with group II control rats (p< 0.001)

REFERENCES

31. Kotnis MS, Patel P, Menon SN and Sane RT. Renoprotective effect of Hemidesmusindicus, a herbal drug used in Cisplatin induced renal toxicity. Nephrology (Carlton), 2004; 9(3); 142-152.