Effect of *Vernonia cinerea* Aerial Parts Against Cisplatin-induced Nephrotoxicity in Rats

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Summary

The present study examined the effect of petroleum ether, ethyl acetate and alcoholic extracts of aerial parts of *Vernonia cinerea* (500 mg/kg, p.o.) on cisplatin-induced nephrotoxicity (6mg/kg, i.p.) in albino rats. Nephroprotector activity of *Vernonia cinerea* was assessed in prophylactic and curative models by estimating blood urea nitrogen, serum creatinine, serum total proteins, urinary protein, creatinine clearance and urine to serum creatinine ratio. Cisplatin elevated blood urea nitrogen, serum creatinine, serum total proteins, increased excretion of urinary protein, decreased the creatinine clearance. Among the three extracts, alcoholic extract showed pronounced curative activity, ethyl acetate extract exhibited good prophylactic activity and petroleum ether extract showed moderate protection in both curative and prophylactic models against cisplatin-induced toxicity.

Keywords: Cisplatin; *Vernonia cinerea* ; Nephrotoxicity ; Nephroprotector activity.

1. Introduction

Cisplatin is a potent anticancer agent with efficacy against a wide variety of tumors but its clinical use is limited due to its nephrotoxicity [1]. Previous reports suggest that free radicals play a major role in the development of nephrotoxicity [2]. Till today there are no drugs available which could effectively prevent the incidence of renal damage or cure the renal damage caused by various agents such as some drugs, industrial/environmental chemicals. Search for nephroprotective agents has resulted in exploration of medicinal plants which were claimed to be useful in the treatment of renal disorders in folklore medicine [3,4].

Vernonia cinerea (L.) Less (F : Compositae) is an annual herb that grows in India. The plant *Vernonia cinerea (V. cinerea)* is used by the village folk of Rayalaseema area, Andhra Pradesh, India, for curing kidney ailments [5,6] . *V. cinerea* is one of the herbs present in the ayurvedic polyherbal preparation, Cystone**.** Cystone was reported to protect kidney against cisplatin-induced toxicity and protection may be mediated through its ability to inhibit lipid peroxidation [7]. The plant was reported to possess anticancer activity against sarcoma 180 in mice [8]. The plant is reported to be used in traditional medicine as tonic, astringent, diaphoretic, antirheumatic, anthelmintic and antidiarrheal [9].

Vernonia cinerea was reported to contain a wide variety of phytochemical constituents having wide range of polarities such as fatty acids, triterpenoids, steroids and flavonoids [10]. In view of the diverse nature of the phytochemical constituents reported in the plant, the present study is aimed to investigate the various extracts of the aerial parts of the plant by using solvents with increasing polarity as the phytoconstituents may be extracted into different solvents based on their polarity. Hence the present study is planned to investigate the nephroprotector activity of Petroleum ether, ethyl acetate and alcoholic extracts of aerial parts of *V. cinerea* on cisplatin-induced nephrotoxicity.

2. Materials and Methods

2.1 Plant material

The aerial parts of *V. cinerea* were collected in the month of November from Tirumala hills, Andhra Pradesh (India), identified by botanist, Sri Venkateswara University, Tirupati and a herbarium specimen has been deposited in the Department of pharmacognosy, Institute of pharmaceutical Technology, Sri Padmavathi university, Tirupati, Andhra Pradesh, India. The plant material was shade dried and powdered.

2.2. Chemicals

Cisplatin was purchased from Sigma Chemical Co., (St. Louis, MO, USA.). Biochemical parameters were estimated by using commercial kits (Ranbaxy Diagnostics, New Delhi, India). All other chemicals used were of analytical grade (S.D. fine or Merck, India).

2.3. Preparation of Various Extracts

The air dried aerial parts (2 kg.) were powdered in a Wiley mill and extracted with petroleum ether (3 Lts x 3), ethyl acetate (5 Lts. x 3) and ethanol (4 Lts. x 3) successively. The above extracts were concentrated under reduced pressure to obtain residues (40, 25, 60 g).

***2.4. Pharmacological studies*:**

2.4.1. Animals*:* The study was performed on Wistar strain albino rats of either sex (120 days) weighing 150-200g. They were maintained on standard diet (Gold Mohar pellets, Bangalore) and water was given *ad libitum*. They were housed in polypropylene cages and were acclimatized to laboratory environment for about a week. The study was conducted after obtaining Institutional ethical committee clearance.

2.4.2. Renal toxicity: Cisplatin was prepared in distilled water to give 1mg/ml solution. To induce nephrotoxicity in rats, cisplatin was administered at 6 mg/kg, intraperitoneally, single dose. Petroleum ether and ethyl acetate extracts were prepared in 1% tween 80 (40mg/ml). The alcoholic extract was prepared in 2% gum acacia (40 mg/ml) and extracts were administered orally by gastric intubation.

2.4.3. Expermental protocol:

2.4.3.1. Effect of petroleum ether, ethyl acetate and the alcoholic extracts of V. cinerea in normal rats*:*

The animals were divided into three groups of six rats in each. Group I received petroleum ether extract (500mg/kg/d p.o.) from day 1 to day 10. Group II received ethyl acetate extract (500mg/kg/d p.o.) from day 1 to day 10. Group III received the alcoholic extract (500mg/kg/d p.o.) from day 1 to day 10. On the next day, blood and urine samples were collected for the estimation of biochemical parameters.

2.4.3.2. Effect of petroleum ether extract of V.cinerea in cisplatin-induced kidney damage in rats*:* The rats were divided into five groups of six animals each. Animals of Group Ia received 1% tween 80 for 10 days (control). Animals of Group IIa received cisplatin (6mg/kg, i.p., single dose) on day 1 and 1% tween 80 from day 6 onwards for 10 days (Curative control (CC)). On the next day, blood and urine samples were collected for the estimation of biochemical parameters.Animals of Group III_P received cisplatin (6mg/kg, i.p., single dose) on day 1 and petroleum ether extract (500mg/kg/d, p.o.) from day 6 onwards for 10 days (Curative activity). On the next day, blood and urine samples were collected for the estimation of biochemical parameters.Animals of Group IVa received 1% tween 80 from day 1 to day 10 and received cisplatin (6mg/kg, i.p., single dose) on day 11(Prophylactic control, (PC)). On day 15, blood and urine samples were collected for the estimation of biochemical parameters. Animals of Group V_P received petroleum ether extract (500mg/kg/d, p.o.) from day 1 to day 10 and received cisplatin (6mg/kg, i.p., single dose) on day 11(Prophylactic activity). On day 15, blood and urine samples were collected for the estimation of biochemical parameters.

2.4.3.3. Effect of Ethyl acetate extract of V.cinerea in cisplatin-induced kidney damage in rats:

The protocol adopted in petroleum ether extract treated model was followed in this study. Ethyl acetate extract (500mg/kg/d p.o.) was used instead of petroleum ether extract (Group III_E (Curative activity), Group V_E (Prophylactic activity).

2.4.3.4. Effect of Alcoholic extract of V.cinerea in cisplatin-induced kidney damage in rats:

The rats were divided into five groups of six animals each. Animals of Group I_b received 1% Gum acacia for 10 days (control). Animals of Group II_b received cisplatin (6mg/kg, i.p., single dose) on day 1 and 1% Gum acacia from day 6 onwards for 10 days (CC). On day 16, blood and urine samples were collected for the estimation of biochemical parameters. Animals of Group III_A received cisplatin (6mg/kg, i.p., single dose) on day 1 and the alcoholic extract (500mg/kg/d, p.o.) from day 6 onwards for 10 days (Curative activity). On day 16, blood and urine samples were collected for the estimation of biochemical parameters. Animals of Group IV_b received 1% Gum acacia from day 1 to day 10 and received cisplatin (6mg/kg, i.p., single dose) on day 11 (PC). On day 15, blood and urine samples were collected for the estimation of biochemical parameters. Animals of Group V_E received the alcoholic extract (500mg/kg/d, p.o.) from day 1 to day 10 and received cisplatin (6mg/kg, i.p., single dose) on day 11 (Group V, Prophylactic activity). On day 15, blood and urine samples were collected for the estimation of biochemical parameters.

2.4.4. Assessment of renal function

Blood urea nitrogen (BUN, Diacetyl monooxime method), Serum creatinine (SC, Alkaline picrate method), Serum total proteins ($S_{TP,}$ Biuret method), Urinary protein (Up, Sulphosalicylic acid method) using UV-Visible spectrophotometer (Systronics) by following standard methods [11]. Creatinine clearance was calculated by using formula:

Creatinine clearance = urinary creatinine X urinary volume h^{-1}/ serum creatinine

2.5. Statistical analysis

The results are expressed as mean±SEM and the data was analysed by one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for *in vivo* studies. Statistical significance was set at $P \leq 0.05$.

3. Results

3.1. Pharmacological studies:

Treatment of petroleum ether, ethyl acetate and alcoholic extracts of *V.cinerea* for 10 days did not cause any significant changes in the levels of serum markers and renal functional parameters when compared with control animals. Hence, the extracts of *V.cinerea* did not show any deteriorative effects on kidney.

3.1.1. Effect of petroleum ether extract on cisplatin-induced renal toxicity: Curative (Group IIa,CC) and prophylactic control (Group IVa,PC) groups were same for petroleum ether and ethyl acetate extracts. Nephrotoxicity was induced by single i.p. injection of cisplatin (6mg/kg). Cisplatin produced significant nephrotoxicity as evidenced by increase in BUN (61% in CC, 68%in PC), SC (62% in CC , 78% in PC), S_{TP} (29% in CC,38%in PC), U_P(61% in CC,66% in PC), reduced Ucr/Scr (55% in CC,56% in PC), Clcr (51% in CC, 50% in PC) when compared with normal control animals. In contrast, animals which belong to Group III_P reduced the levels of serum markers (BUN 45%, SC 23%, S_{TP}17%),U_P (31%), increased Ucr/Scr (36%), Clcr (30%). In prophylactic group (Group V_P) also, the petroleum ether extract exhibited protection against cisplatin-induced effects indicated by decreased levels of BUN (11%), SC (43%), S_{TP} (2%), U_P(19%) and increased Ucr/Scr (7%), Clcr (21%).

3.1.2. Effect of Ethyl acetate extract on cisplatin-induced renal toxicity: Animals which belong to Group III_E prominently decreased the levels of BUN (55%), SC (39%), S_{TP}(20%),U_P(43%) and increased Ucr/Scr (41%), Clcr (31%). Animals which received prophylactic treatment of ethyl acetate extract (Group V_E) were found to prevented significantly all the effects which are developed by cisplatin namely decreased BUN (60%), SC(74%), S_{TP}(36%),U_P(44%) and increased Ucr/Scr (44%), Clcr (28%).

3.1.3.Effect of alcoholic extract on cisplatin-induced renal toxicity: In this study, the effect of alcoholic extract on renal function was examined in cisplatin- induced nephrotoxicity in curative and prophylactic models. Intraperitoneal administration of cisplatin induced nephrotoxicity. It was evidenced by increased levels of BUN (62% in Group II_b, 77% in Group IV_b), SC(58% in Group II_b, 80% in Group IV_b), S_{TP} (17% in Group II_b, 43% in Group IV_b), U_P (64% in Group II_b, 63% in Group IV_b) and reduced the levels of Ucr/Scr (54% in Group II_b, 60% in Group IV_b) and Clcr (30% in Group II_b, 8% in Group IV_b). Animals which received curative treatment significantly inhibited the rise of BUN (61%), SC (58%), S_{TP} (23%),U_P(48%), and increased Ucr/Scr (42%), Clcr (43%).On the other hand, animals which received prophylactic treatment exhibited no alteration in serum markers levels, renal functional parameters levels when compared with animals which received cisplatin alone i.e., prophylactic control.

4. Discussion

Cisplatin is an important anti-tumor agent but, nephrotoxicity is a dose limiting factor in clinical use of cisplatin. Earlier reports suggest that cisplatin exhibits site specific nephrotoxic effect on the S_3 segment of proximal tubule located in the outer stripe of the medulla. One possible mechanism for selective necrosis at S_3 region of the proximal tubule is that cisplatin accumulates in this segment preferentially by secretion. Another possibility is cisplatin exerts its effect indirectly through alteration in renal hemodynamics [12].

The pathological changes were most prominent 3 days after the administration of cisplatin, predominant pattern of injury was observed after 5days [12]. Hence, in

curative regimen the extracts were administered on day 6, i.e., after induction of nephrotoxicity. In curative regimen, the extracts showed significant activity and the order of protection observed was alcoholic > ethyl acetate > petroleum ether extract. Previous reports on phytochemical studies on roots and the whole plant petroleum ether and n-Hexane extracts of aerial parts of *V. cinerea* revealed the presence triterpenoids such as α-amyrin, β-amyrin, lupeol and their derivatives [13-16]. Petroleum ether extract of the roots of *V. cinerea* was reported to contain steroids like stigmasterol and their derivatives [14]. Ethanolic extract of whole plant of *V. cinerea* was reported to contain phenolic resins [16]. Based on these results, the promising activity observed with the alcoholic extract may be presence of phenolic resins.

Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules [17]. Because this renal damage occurs in the first hour after administration, it is important that the protective agent is present at sufficient concentrations in renal tissue before the damage occurs [18, 19]. This is the rationale behind the prophylactic treatment. Ethyl acetate extract effectively protected the cisplatin induced renal damage. Petroleum ether extract exhibited partial but significant protection, where as the alcoholic extract failed to prevent the effects that are developed by cisplatin. It is interesting to note that the alcoholic extract which exhibited promising curative activity failed to show any prophylactic activity. The varied biological activity with the three extracts of *V.cinerea* in cisplatin-induced nephrotoxicity might be attributed to the presence of different phytochemical constituents in these extracts and also probably due to different mechanisms involved in the prophylaxis and cure of nephrotoxicity.

Various reports suggested that several plants containing antioxidant principles such as *pongamia pinnata, crataeva nurvala* exhibited nephroprotector activity against cisplatin-induced renal damage [3, 20]. Further, a triterpenoid lupeol which was isolated from *crataeva nurvala* and flavonoids such as quercetin, luteolin were also exhibited protection against cisplatin-induced kidney damage[21,22]. Tannis are well known to possess antioxidant action [23]. Flavonoids exhibit several biological activities including antioxidant and free radical scavenging abilities [24]. A relationship between oxidative stress and renal toxicity has been well documented in many experimental animal models. *V.cinerea* reported to contain luteolin and pentacyclic triterpenoids, phenolic resins [13-16]. Hence the presence of these constituents in *V. cinerea* may be responsible for the nephroprotector activity.

As the present study was planed to evaluate the different extracts of *V.cinerea* at single dose i.e., 500mg/kg. All three extracts were exhibited good activity against cisplatin-induced renal damage and these extracts were showed prominent affect on serum markers levels then on renal functional parameters. The results of present study revealed the promising curative activity by alcoholic extract and significant prophylactic activity by ethyl acetate extract. Hence, the alcoholic and ethyl acetate extracts may have scope to evaluate nephroprotector activity further at lower doses.

The results of the present study substantiate the use of *V. cinerea* in the form of polyherbal ayurvedic preparation (cystone) and the claimed use of it in folklore medicine in various renal disorders.

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