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Nephroprotective activities of root extracts of *Andrographis paniculata (Burm f.) Nees* in gentamicin induced renal failure in rats: A time-dependent study

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Abstract

Kidney Disease is an increasingly common condition with limited treatment options placing a major financial and emotional burden on the community. There is emerging evidence in the literature about renoprotective complementary and alternative medicines. A primary goal of this article is to present the scientific evidence for the use of herbs like Andrographis paniculata (AP) as a complementary treatment for acute renal failure (ARF). Wistar rats were divided as follows: Normal, Gentamicin (GM) and Root extracts (Pt. ether, $CHCl_3$ and MeOH) of AP (200 mg/kg; p.o.) treated groups. The nephrotoxic model was prepared by GM (80 mg/kg; i.p. × 8 days). The degree of nephroprotection was measured by using renal parameters like serum creatinine (SCr), serum urea (SU) and urinary proteins (UP) after 3, 7 and 10 days of ingestion of various extracts. Oral administration of Pt. ether ($p<0.05$), $CHCl_3$ ($p<0.01$) and MeOH ($p<0.001$) extracts of AP patently prevented gentamicin induced elevated levels of SCr, SU and UP. The results were also supported by measuring the urine volume voided by each rat separately, of all the groups with time. The extent of protection offered by various extracts under study increased with the increasing time of treatment and polarity of the solvents. The signs of GM nephrotoxicity in rats are significantly mitigated by Pt. ether and $CHCl_3$ extracts whereas the maximal alleviation of ARF was caused by MeOH root extract; hence, the methanolic root extract of AP can be advocated as a nephroprotective agent.

Keywords: Acute renal failure, *Andrographis paniculata*, gentamicin, root extract.

Introduction

Renal failure is a common clinical syndrome. It is defined as a rapid decline in renal function resulting in abnormal retention of serum creatinine and blood urea which must be excreted. The clinical manifestations of renal failure are the decline in glomerular filtration rate (GFR) and the inability of the kidney to excrete the toxic metabolic substances produced in the body. Kidney disease is the ninth leading cause of death in United States [1]. Approximately, 19 million United States adults have chronic kidney disease and an estimated 80,000 persons have chronic kidney failure diagnosed annually [2]. The incidence of End Stage Renal Disease (ESRD) is increasing, with a doubling in the number of patients treated for ESRD seen in the United States and China over the past decade [3, 4]. Two community based studies, although methodologically different, have shown a prevalence of chronic renal failure of 0.16% and 0.79% in India [5]. Till date for End Stage Renal Failure, renal replacement is the only therapy. In case, of non-availability of kidney, dialysis is the only alternative, which unfortunately is severely limited by several constraints including a good amount of expenditure. No exclusive drug has been reported so far, as such in any category of medical treatment. The worldwide rise in the number of patients with chronic kidney disease (CKD) and consequent end-stage renal failure necessitating renal replacement therapy is threatening to reach epidemic proportions over the next decade, and only a small number of countries have robust economies able to meet the challenges posed. A change in global approach to CKD from treatment of ESRD to much more aggressive; primary and secondary prevention is therefore imperative [6]. Today popularity of complementary medicine has increased, Worldwide. Herbal remedies have been developed by traditional knowledge of herbs, which is a ray of hope for kidney failure patients. A number of herbs, traditionally used are *Rheum palmatum* [7], *Cinnamomum cassia* [8], *Panax ginseng* [9], Astragalus *membranaceus* [10], *Chinese rhubarb* [11], *Centella asiatica* and *Capsicum spp.* (cayenne) [12].

Andrographis paniculata (Burm. F.) Nees (Acanthaceae) commonly known as ‘Kalmegh’ is used as a bitter ingredient in 26 Ayurvedic formulations as immunomodulatory [13], antiangiogenic [14], anticancer [15] and in treatment of various liver disorders [16]. However, no attention has been paid so far to explore its nephroprotective activity in animals and human beings. In continuation of our work exploring herbal potential for diabetes [17, 18] and kidney failure [19], the present paper explores the potential of root extracts (Petroleum ether, Chloroform and Methanol) of *Andrographis paniculata* for significant amelioration of renal failure in gentamicin induced nephrotoxic Wistar male albino rats.

Materials and Methods

Plant Material

Authenticated fresh plants of *Andrographis paniculata* were obtained from National Botanical Research Institute, Lucknow, India and were washed and root parts were separated. The shade dried root parts were powdered and finally stored in air tight glass jars, separately.

Preparation of Extract

The powdered material (roots) subjected to successive Soxhlation with solvents of increasing polarity (Pt. ether, $CHCl_3$ and MeOH). The filtrate was evaporated to dryness under reduced pressure. The semi solid mass thus obtained was suspended in 1% gum acacia. Test extract (1 ml) equivalent to dose of 200 mg/kg body weight was administered to rats. The suspension was thoroughly mixed to ensure homogeneity prior to administration.

Experimental Animals

Male Wistar albino rats weighing 130±10 g were housed in polypropylene cages and maintained at 24 ± 2°C under 12 hour light/dark and 60 ± 5 % humidity. They were fed with Amrut Laboratory Animal Feed, manufactured by Nav Maharashtra Chakan Oil Mills Ltd., Pune, India. Water was provided ad libitum. The animals were acclimatized for a week under laboratory conditions. All experiments were performed according to the norms of the local ethical committee.

Experimental Design

Experimental animals were distributed randomly, in five groups, containing six animals each.

a. Normal animals

Group I received vehicle only, throughout the experimental period.

b. Nephrotoxic animals

The animals were injected with Gentamicin (Wockhardt Ltd., India), intraperitoneally at a dose of 80 mg/kg for eight consecutive days at the 8:00 hrs in the morning to induce nephrotoxicity [20, 21]. These nephrotoxic animals were divided into four groups. Group II received vehicle only and group III, IV, V, received Pt. ether, $CHCl_3$ and MeOH root extracts, daily at a dose of 200 mg/kg (p.o.), respectively, for 10 consecutive days. The serum creatinine, serum urea, urinary proteins and urine volume were measured on 3^{rd}, 7^{th} and 10^{th} day of the treatment.

Sample Collection

Individual rats belonging to different groups were placed in metabolic cages over a period of 24 h and urine was collected. At the end of 24 hours, rats were anesthetized with a combination of ketamine (60mg/kg) and xylazine (5mg/kg) given intraperitoneally. Blood samples were collected via retro orbital puncture in plain plastic tubes, left to stand at 4 °C for 1 hour, and centrifuged (900 × g for 15 min at 5 °C) to separate serum. The serum obtained was stored at - 5 °C until analysis.

Biochemical Analysis

Plasma and urine samples were assayed using standard diagnostic kits, viz. serum creatinine (Human, Germany), serum urea (Beacon Diagnostics, India) and urinary protein (ERBA Diagnostics, Germany).

Statistical Analysis

All values were expressed as mean ± standard error. Differences within groups were evaluated by paired *t*-test. One-way analysis of variance was used for examining differences among groups. Inter-group comparisons were made with Dunnet's multiple-comparison test. A p-value of < 0.05 was considered to indicate significance.

Results

The Pt. ether, $CHCl_3$ and MeOH extracts of *A. paniculata* roots produced a time and solvent dependent nephroprotection in gentamicin-induced nephrotoxic rats.

Effect on Serum creatinine

Table 1 demonstrates a significant reduction in serum creatinine with *A. paniculata* root extracts viz. Pt. ether (28.66%, 3d, $p<0.05$), (46.30%, 7d, $p<0.001$), 46.68%, 10d, $p<0.01$); $CHCl_3$ (38.90%, 3d, $p<0.01$), (57.41%, 7d, $p<0.0001$), (59.88%, 10d, $p<0.0001$) and MeOH (50.24%, 3d, $p<0.0001$), (65.38%, 7d, $p<0.0001$) and (72.82%, 10d, $p<0.0001$) as compared to gentamicin

control. Percentage of reduction increases with the increasing time and maximum nephroprotection was recorded in methanolic root extract at day 10.

Table 1: Effect of *Andrographis paniculata* root (200 mg/kg) on serum creatinine (mg/dl) in nephrotoxic rats

Groups	Serum Creatinine			
	0 day	**3 days**	**7 days**	**10 days**
Normal	0.260 ± 0.03	0.250 ± 0.02	0.258 ± 0.02	0.264 ± 0.02
Gentamicin Control	1.554 ± 0.34$^{\#\#}$	1.640 ± 0.18$^{\#\#}$ (5.53)	1.404 ± 0.06$^{\#\#}$ (-9.65)	1.376 ± 0.06$^{\#\#}$ (-11.45)
Pt. ether	1.580 ± 0.36	1.170 ± 0.21^{a} (-25.95)	0.754 ± 0.06*^{c} (-52.28)	0.742 ± 0.06 *^{d} (-53.04)
$CHCl_3$	1.916 ± 0.15	1.002 ± 0.64 § (-47.70)	0.598 ± 0.07 $^{\square\,d}$ (-68.79)	0.552 ± 0.17 $^{\square\,d}$ (-71.19)
MeOH	2.01 ± 0.39	0.816 ± 0.08 $^{\#d}$ (-59.40)	0.486 ± 0.07 $^{\square\,d}$ (-75.82)	0.374 ± 0.07 $^{\square\,d}$ (-81.39)

Values are mean ± SE of 6 rats
Figures in parenthesis indicate percent change in Serum Creatinine level with respect to 0 day.
3, 7, 10 and 15 days are compared with 0 day § p<0.05; *p<0.01; $^{\#}$p<0.001; $^{\square}$ p<0.0001
$^{\#\#}$p<0.0001 vs. normal; ^{a}p<0.05; ^{b}p<0.01; ^{c}p<0.001; ^{d}p<0.0001 vs. gentamicin control.

Effect on Serum Urea

Maximal reduction of serum urea with Pt. ether (43.04%, p<0.01), $CHCl_3$ (63.40%, p<0.0001) and MeOH (68.00%, p<0.0001) was observed after 7 days, when compared to gentamicin control (Table 2). The amelioration in nephrotoxicity was insignificant at day 10 when compared to day 7.

Table 2: Effect of *Andrographis paniculata* root (200 mg/kg) on serum urea level (mg/dl) in nephrotoxic rats

Groups	Serum Urea			
	0 day	**3 days**	**7 days**	**10 days**
Normal	19.38 ± 0.36	19.26 ± 0.41	19.31 ± 0.34	19.22 ± 0.35
Gentamicin Control	63.10 ± 3.90	71.74 ± 3.58 (13.69)	67.60 ± 4.60 (7.13)	60.72 ± 4.21 (-3.77)
Pt. ether	73.92 ± 3.19	47.39 ± 7.76$^{\S a}$ (-35.89)	38.50 ± 4.18$^{\# b}$ (-47.9)	37.77 ± 4.29$^{\#}$ (-48.90)
$CHCl_3$	90.13 ± 12.07^{a}	41.82 ± 7.08*^{b} (-53.60)	24.74 ± 1.21$^{\# d}$ (-72.55)	24.27 ± 1.15$^{\# d}$ (-73.07)
MeOH	92.16 ± 4.23^{b}	28.72 ± 3.18$^{\dagger d}$ (-68.84)	21.63 ± 2.01$^{\dagger d}$ (-76.53)	20.16 ± 1.51$^{\dagger d}$ (-78.13)

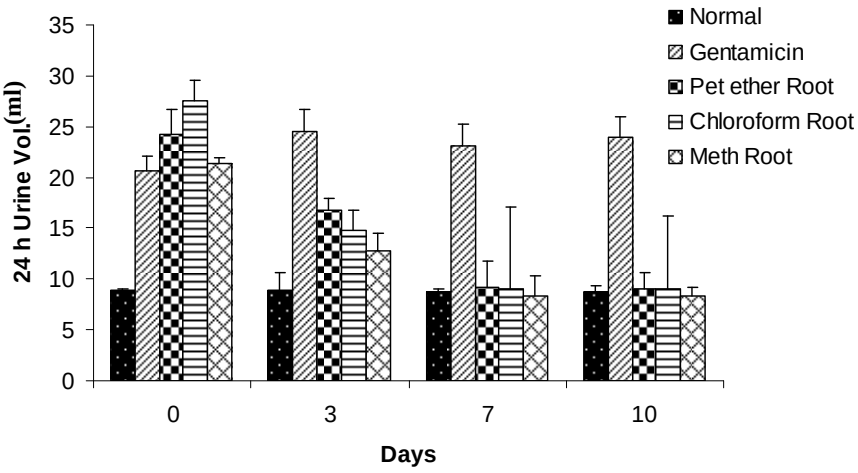
Effect on Urinary Proteins

Table 3 depicts the reversal of the nephrotoxicity in terms of the urinary secretions of proteins in various root extracts treated rats. The order of renoprotection was found to be 11.50% with Pt. ether, 21.69% with $CHCl_3$ and 30.47% with MeOH root extracts, after 7 days of the treatment and the percent reduction was levelled off at day 10.

Table 3: Effect of *Andrographis paniculata* root (200 mg/kg) on urinary proteins level (g/100 ml) in nephrotoxic rats

Groups	Urinary protein			
	0 day	**3 days**	**7 days**	**10 days**
Normal	5.69±0.07	5.62±0.08	5.68±0.05	5.72±0.07
Gentamicin Control	9.93±0.11	9.96±0.12 (0.30)	9.91±0.06 (-20.00)	8.92±0.28* (-10.17)
Pt. ether	9.60±0.23	8.99±0.27[a] (-6.35)	8.77±0.24[§b] (-8.65)	8.69±0.26[§] (-9.48)
$CHCl_3$	9.86±0.19	8.58±0.32*[b] (-12.98)	7.76±0.31[# c] (-21.29)	7.63±0.26[# b] (-22.62)
MeOH	9.95±0.29	7.91±0.22[# d] (-20.50)	6.89±0.36[# d] (-30.75)	6.79±0.37[# b] (-31.76)

Figure 1: Effect of *Andrographis paniculata* root extracts on 24-h urine volume (ml) in nephrotoxic rats



Each column and bar represents the mean ± S.D. of 6 animals, *p<0.05 vs. Normal

Effect on Urine Volume

Mean urine volumes were significantly elevated in gentamicin control rats throughout the experimental period, while *A. paniculata* root extracts treated rats excreted urine volumes within the normal range. Results are shown in Fig 1.

Discussion

In the present study, an effort has been made to examine the nephroprotective ability of different root extracts of *Andrographis paniculata* as a function of time and the polarity of solvents used. It is evident from the data that the effectiveness of the extracts improved with the polarity of the solvents over a period of 10 days.

Our results clearly indicate that the gentamicin, at a dose of 80 mg/kg/day produces nephrotoxicity as evidenced by the increased serum creatinine and serum urea suggesting impairment in glomerular function. Serum creatinine concentration is a more significant indicator than the blood urea concentration at an earlier phase of kidney disease [22]. *Andrographis paniculata* Nees (Acanthaceae) has a long history of use in traditional forms of oriental medicine and it has enjoyed popular use in India for a century. In addition, to its well known hepatoprotective activity, the plant has strong ability to remove reactive oxygen species (ROS) [23] and gentamicin nephrotoxicity involves generation of ROS [24]. The beneficial effect of *A. paniculata* against gentamicin nephrotoxicity possibly depends on its ability to scavenge the gentamicin induced free radicals. The mechanism(s) remains to be elucidated.

Our results strongly suggests that *A. paniculata*, at a dose used, was effective in mitigating the biochemical and physiological changes induced by gentamicin. Support for the salutatory action on the renal function was obtained from the alleviation of the increase in serum concentrations of creatinine and urea. The analysis of urine volume in this work confirmed that gentamicin caused a polyuric acute renal failure, as judged by significant increase in the volume of urine voided and concomitant with a significant increase in protein concentration.

The effects induced by gentamicin were significantly reversed, albeit not completely by Pt. ether and $CHCl_3$ extracts but almost closer to the normal by MeOH extract of *Andrographis paniculata* root, adding further evidence that the plant has the potential to ameliorate gentamicin nephrotoxicity. This is the first report, as far as we are aware, on the renoprotective effect of *Andrographis paniculata*.

Conclusion

In conclusion, the study suggests that *Andrographis paniculata* root extracts has the potential to mitigate the signs of gentamicin nephrotoxicity in rats. The maximal amelioration of acute renal failure was observed in methanolic extract after 10 days of the treatment, and hence, the methanolic extract of *Andrographis paniculata* roots can be advocated as a nephroprotective agent.

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