

Nephroprotective Potential Compounds from Leaves Extracts of *Andrographis paniculata*

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Abstract

*Nephrotoxicity is the third most common problem of the renal system. Medicinal plants potentially useful for the treatment of severe renal disorders. In the present study, the effect of aqueous, ethanol and acetone extract of *Andrographis paniculata* leaves was evaluated by pretreating three groups of rat animals. Renal failure was induced by antibiotic drug gentamicin which was orally administered to group II animals and untreated control model was maintained. After oral administration for 10 days, serum levels of urea, uric acid, creatinine and total protein were assayed. All the biochemical parameters were significantly controlled ($p < 0.01$ and $p < 0.001$) on 10th day after the treatment of aqueous, ethanol and acetone extracts as compared than the gentamicin induced nephrotoxic animals ($p < 0.05$). Maximum nephroprotection was offered by the ethanol extract of *A. paniculata* leaves ($p < 0.001$). The histological structure of renal was observed by staining with haematoxylin- eosin viewed using light microscope. Gentamicin treated animals showed acute tubular necrosis due the injury in kidney. In aqueous, ethanol and acetone extract treated animals showed histopathological changes in renal revealed the nephroprotective activity of *A. paniculata* leaves. Finally, ethanol extract of *A. paniculata* was more suitable for nephroprotective action against gentamicin induced renal failure was evidenced by biochemical estimation and by restoration of histological changes of renal system.*

Keywords: Kidney, Nephroprotection, *Andrographis paniculata*, Herbal Medicine.

Introduction

Kidney is the complex and major organ of our body perform several important functions like formation of urine, water and salt metabolism, acid-base balance, regulation of blood calcium level^[1] and secretion of hormones.^[2] Kidney affected by the diseases are mainly kidney blockage and kidney stones. Major types of stones in kidney are calcium stones, stuvite stones, uric acid stones and cysteine stones.^[3] Acute renal failures and chronic renal failure are common and serious problems. Acute renal failure is reversible loss of kidney function whereas chronic renal failure is irreversible loss of kidney function.^[4]

Nephrotoxicity is one of the major kidney problems caused by drug or toxin^[5] Drugs, diagnostic agents, chemical reagents and heavy metals are well known to be nephrotoxic.^[6] In recent years, development of modern medical, and surgical practices has been followed for the treatment of renal failure like haemodialysis, renal transplantation and chemotherapy. These procedures are complicated and high cost has been utilized to cure the kidney damage. So that the traditional medicine using herbal plant is best method than the conventional method. However, when using these chemotherapy method may induce the side effects to the body.^[7] Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Drugs like gentamicin, cisplatin, cyclosporine, Carbon tetrachloride are common source of acute kidney injury. Gentamicin is an amino glycoside antibiotics used for the treatment of Gram negative bacterial infections. Overdose of gentamicin causes renal damage. It may give serious side effects while continuous consuming at higher concentrations.

Medicinal plants are have curative properties and therapeutic values due to the presence of various complex phytochemical compounds. This traditional medicines are assuming greater important because of very effective, safer, locally available, and no side effects.^[8, 9] *A. paniculata*, a member of the family of Acanthaceae is locally and commonly available plant in India. It is commonly called as “nilavembu or siriyanangai” in Tamil and “King of bitters” in English. It has been shown hepatoprotective, anti-

parasitic, antioxidant anti-inflammatory and antimicrobial activity. And used helps in malaria treatment and treatment of cancer.^[10] In this study reported that curative properties of the medicinal plant *Andrographis paniculata* against nephrotoxicity induced by gentamicin in albino rats. Nephroprotective activity was confirmed by examine biochemical tests for urea, uric acid creatinine and protein level in blood and histopathological studies carried out using light microscopic observations.

Materials and methods

Preparation of plant leaf extracts

Leaves of *Andrographis paniculata* were collected and shade dried for 3-5 days and grinded into powder. Aqueous extract was prepared by adding dried powder into 100 ml water and incubate overnight. 100 g dry powder was extracted with 80 % ethanol at 55°C for 24 hours in soxhlet apparatus. Acetone extract was prepared by mixing of dried leaf powder with the 80% acetone. Solvent elimination was done at room temperature and stored. The resulting aqueous, ethanol and acetone extracts were then used for nephroprotective activity.

Experimental Design for nephroprotective Activity of *Andrographis paniculata*

Adult male Wister albino rats maintained at the college weighing between 150g-170g were used for the nephroprotective studies. Animals were divided into six groups in six rats each. **Group I (Normal):** Orally received distilled water for 10 days.

Group II (Induced): Orally received Gentamicin (80 mg/kg body weight) only for 10 days. **Group III (Standard):** Orally received Cystone (20 mg/kg body weight) along with gentamicin (80mg/kg body weight) for 10 days.

Group IV (Treatment): Orally received aqueous leaf extract (300mg/kg body weight) along with Gentamicin (80mg/kg body weight) for 10 days.

Group V (Treatment): Orally received ethanol leaf extract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Group VI (Treatment): Orally received acetone leaf extract (300mg/kg body weight) along with Gentamicin (80 mg/kg body weight) for 10 days.

Cystone was used as positive control for comparing nephroprotective potential of different leaves extract of *A. paniculata*. Gentamicin is act as nephrotoxin which induces the kidney damage.

Histopathological and biochemical study

After 10 days, all animals from every group were sacrificed and separated the kidneys by dissection procedure. Pieces of kidneys obtained from each group were immediately fixed in 10% formalin solution. The fixed formalin fixed kidneys were embedded in paraffin and serial section were made and stained with haematoxylin and eosin. The stained sections were examined under light microscope. Blood samples were collected from jugular vein. Serum was separated from the blood for the analysis of the parameters like Blood Urea^[11], Uric Acid^[12], Creatinine^[13] and Total Protein.^[14]

Statistical analysis

Data were analyzed using one way Analysis of Variance (ANOVA) and expressed as mean \pm S.E.M. Statistical significance was fixed $p < 0.05$.

Results and discussion

Biochemical studies

The nephroprotective activity of aqueous, ethanol and acetone extract of *A. paniculata* leaves was assessed against nephrotoxicity induced using gentamicin in albino rats. The nephroprotective activity was determined by biochemical tests and histopathological studies. Table 1 shows the changes of urea and uric acid level in blood. Blood urea and blood uric acid in the control (group I) was estimated to be 30.16 ± 1.72 mg/dl and 5.08 ± 0.21 mg/dl, respectively. In the negative control i.e. group II animals received only gentamicin which shows level of urea and uric acid in blood to be 59.00 ± 2.19 mg/dl and 8.25 ± 0.54 mg/dl, respectively. Group IV, V and VI animals received gentamicin along with aqueous leaf extract, ethanol and acetone extract demonstrated a significant increase ($p < 0.05$ to $p < 0.001$) in

blood urea and uric acid as compared with negative control group. In the ethanol treated groups shows most significant changes ($p < 0.001$) in urea and uric acid recorded as 33.28 ± 0.54 mg/dl and 5.21 ± 0.12 mg/dl as compared with aqueous and acetone extract (Figure 1).

Creatinine concentrations in blood was significantly increased ($p < 0.05$) in the gentamicin treated negative control group of animals (2.88 ± 0.11 mg/dl) compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with plant extracts of *A. paniculata* showed significant ($p < 0.01$ and $p < 0.001$) increase in creatinine concentrations. Ethanol extract treated animals showed increased significant changes ($p < 0.001$) recorded as 0.82 ± 0.54 mg/dl concentrations of creatinine, indicates that nephrotoxicity curative properties of *A. paniculata* leaves (Table 2).

Normal total protein level was observed in group I animals. Gentamicin treated group II animals showed low amount of secretion of total proteins ($p < 0.05$) as compared to normal animals. This low protein level in serum is probably due to an inhibitory action of protein synthesis induction of tissue damage and may leads to increased excretion of protein in urine (Ramesh et al 2014). Treatment with the plant extracts of *A. paniculata* (group IV, V and VI) showed ($p < 0.01$ and $p < 0.001$) increase in concentrations of total protein compared to the gentamicin treated groups (group II) (Table 2, Figure 2).

This inhibitory action of *A. paniculata* leaves extract against nephrotoxin was confirmed through biochemical and histopathological studies. This activity may due to the presence of secondary metabolites like flavonoid and polyphenolic compounds which may be responsible for the kidney protective activity.

Figure captions

Table 1. Effect of aqueous, ethanol and acetone extract of *A. paniculata* leaves on blood urea and uric acid in gentamicin induced nephrotoxic rats

Parameters	Blood Urea(mg/dl)	Blood Uric acid (mg/dl)
Group I (Normal)	30.16 ± 1.72	5.08 ± 0.21
Group II (induced)	$59.00 \pm 2.19^*$	$8.28 \pm 0.54^*$
Group III (Standard drug)	$34.83 \pm 3.06^{**}$	$5.95 \pm 0.21^{**}$
Group IV (Aqueous)	$36.16 \pm 2.48^{**}$	$6.12 \pm 0.33^{**}$
Group V (Ethanol)	$33.28 \pm 0.54^{***}$	$5.21 \pm 0.12^{***}$
Group VI (acetone)	$39.08 \pm 0.21^{***}$	$6.36 \pm 0.13^{***}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ value are considered statistically significant (BMRT)

Table 2. Effect of aqueous, ethanol and acetone extract of *A. paniculata* leaves on creatinine and total protein levels in gentamicin induced nephrotoxic rats

Parameters	Creatinine (mg/dl)	Total protein (mg/dl)
Group I (Normal)	0.79 ± 0.02	6.91 ± 0.59
Group II (induced)	$2.88 \pm 0.11^*$	$3.02 \pm 0.84^*$
Group III (Standard drug)	$1.21 \pm 0.12^{**}$	$7.55 \pm 0.70^{**}$
Group IV (Aqueous)	$1.11 \pm 0.13^{***}$	$7.12 \pm 0.77^{**}$
Group V (Ethanol)	$0.82 \pm 0.54^{***}$	$7.91 \pm 0.12^{***}$
Group VI (acetone)	$1.38 \pm 0.21^{**}$	$7.56 \pm 0.13^{***}$

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ value are considered statistically significant (BMRT)

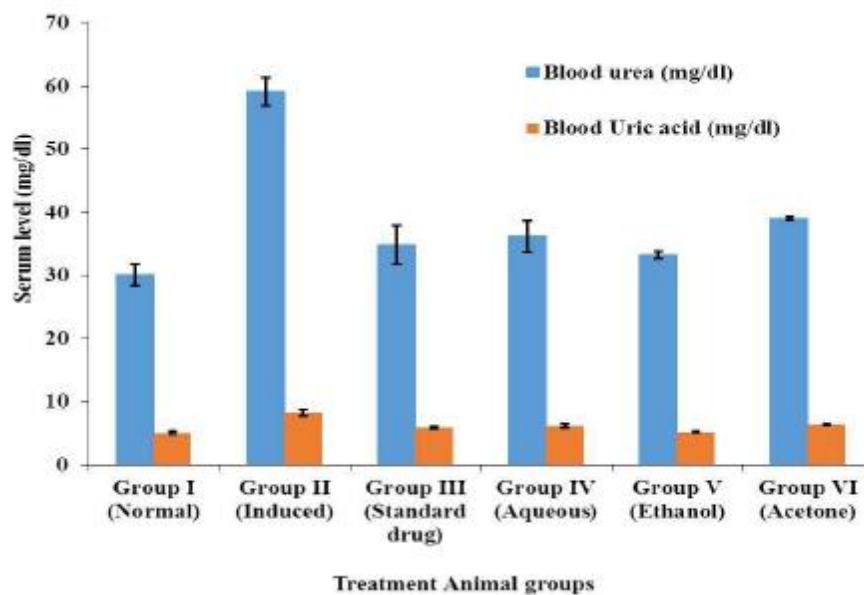


Figure 1. Effect of aqueous, ethanol and acetone *A. paniculata* leaves extract on the alterations of urea, and uric acid level in blood

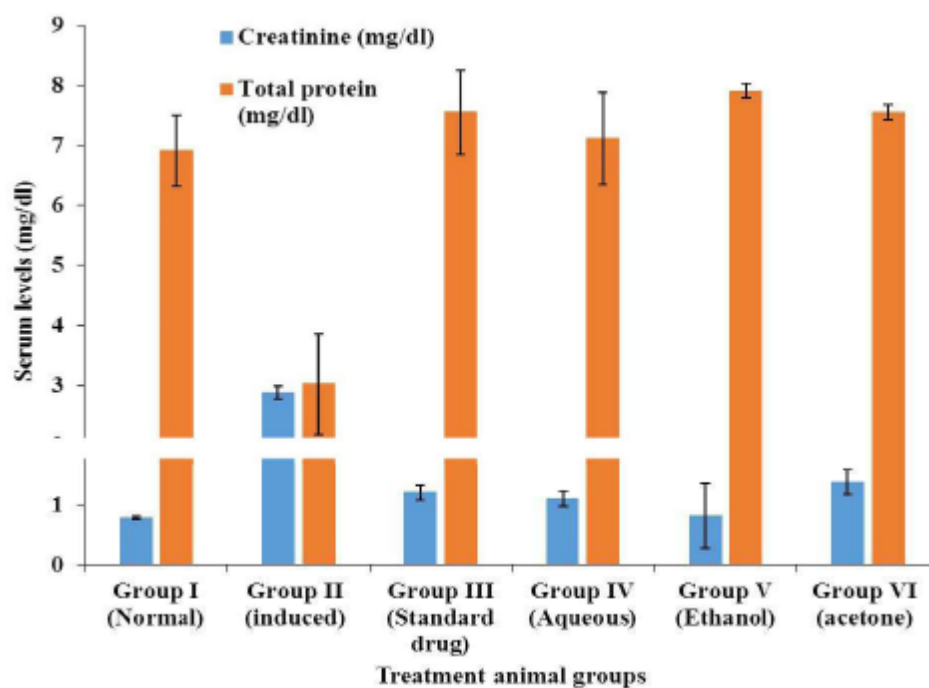


Figure 2. Effect of aqueous, ethanol and acetone *A. paniculata* leaves extract on the alterations of creatinine, and total protein level in blood

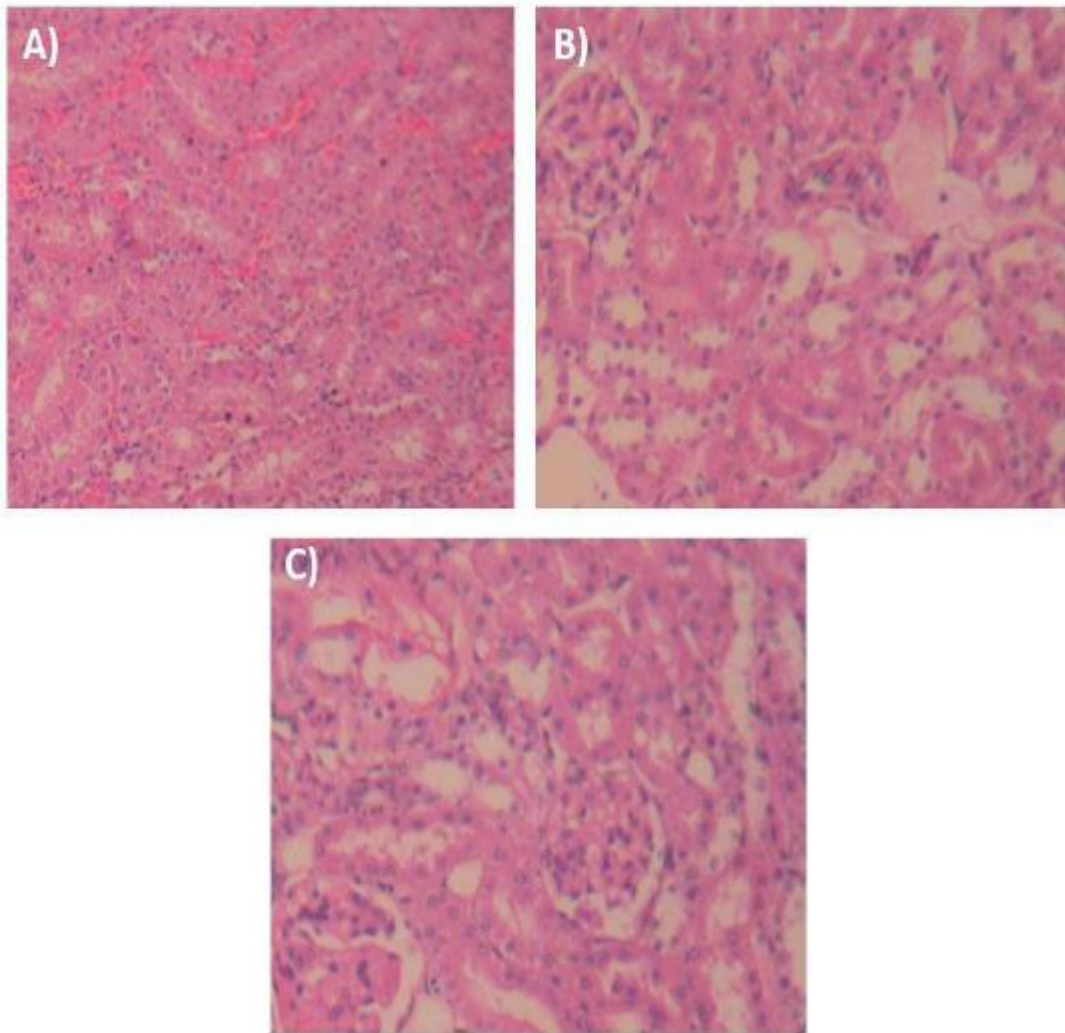


Figure 3. Sectioning of (A) normal kidney showing tubular brush borders and intact glomeruli in renal tissues without alterations (B) Representing the tubular necrosis in gentamicin treated animals (C) shows microscopic observation of normalized kidney structure on treated with cysteine is a positive control

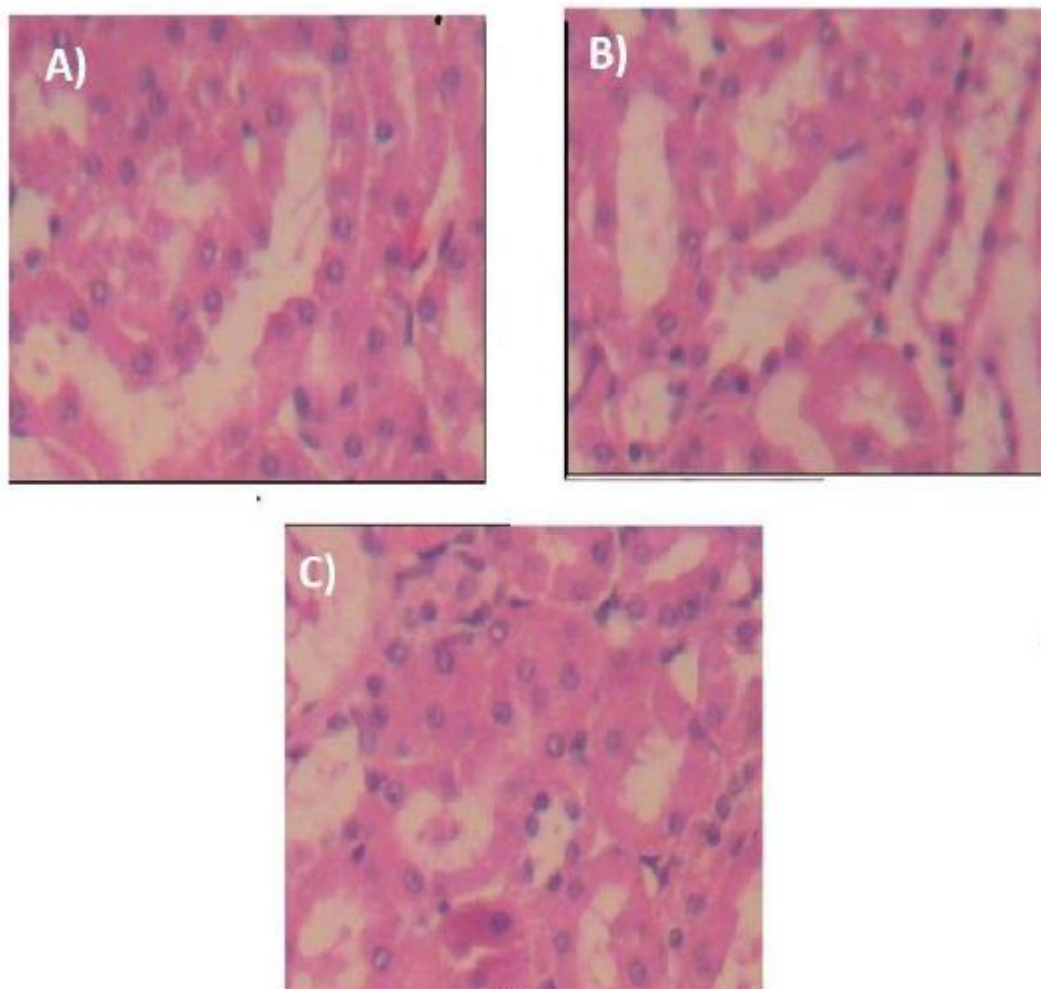


Figure 4. Shows histological structure of kidney treated with (A) aqueous extract (B) ethanol extract (C) acetone extract of *A. paniculata* leaves on gentamicin induced nephrotoxicity rat animals revealed normalized and restored function of renal system

Conclusion

Herbal medicine have useful for the development of effective therapy to treat variety of diseases. Nephrotoxicity induced by gentamicin in rats developed significant kidney injuries was estimated from increased levels of urea, uric acid, and creatinine ($p < 0.05$) and decreased levels of total protein in blood serum. These parameters were more significantly ($p < 0.001$) altered and restored by oral supplementation of aqueous, ethanol and acetone extract of *A. paniculata* leaves to gentamicin intoxicated rats. Our present study clearly indicated a significant nephroprotective activity by normalize the elevated biochemical and restoration of renal system using with the extract of *A. paniculata* leaves and supported the traditional usage of the plant in the medicinal system.

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