Evaluating the nephron protective activity of *Argyreia nervosa* wholeplant against gentamicin induced nephrotoxicity in albino Wister rats

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**Abstract**

**Objective:** The objective of the present study is to develop the pharmacological activity of the herbal drug using ethanolic extract of whole plant of *Argyreia nervosa* which is claimed for its effective treatment of ailments with low toxicity and economic viability. Collection and authentication of whole plant *A. nervosa*. Extraction of leaves of *A. nervosa* with 90% ethanol. Phytochemical screening of ethanolic extract of *A. nervosa* whole plant. Pharmacological evaluation of ethanolic extract biochemical parameters such as estimation of levels of creatinine in blood serum, estimation of levels of urea in blood serum, estimation of levels of uric acid in blood serum and Statistical analysis.

**Materials and Methods:** Picric acid, alkalin buffer reagent, standard creatinine, surfactant, urea reagent, acid reagent, diaceylmonoxime reagent, urea standard, buffer reagent, enzyme reagent, uric acid standard, *A. nervosa*, gentamicin, di-ethyl ether. Tests for flavonoids, tests for steroids, tests for alkaloids, tests for tannins, tests for carbohydrates, tests for cardiac glycosides, tests for anthra quinones glycosides, tests for amino acids, test for phenols, acute oral toxicity studies, experimental design: The animal experiments were performed after acclimatization animals were separated in to four groups of each group with 6 animals were placed in separate cages, collection of blood, estimation of serum creatinine, estimation of uric acid, estimation of urea, statistical report.

**Conclusion:** The presence of flavonoids, tannins, steroids, carbohydrates, phenols etc. In acute toxicity studies the test dose 200 mg/kg and 400 mg/kg was chosen as experimental doses. Nephroprotective activity of ethanolic extract of *A. nervosa* was screened for biochemical parameters (serum creatinine, serum uric acid, serum urea). The ethanolic extracts have produced effective nephroprotective action as evident by results obtained from the studies. The interpretation of the results was done after subjecting the data obtained from various studies by simple graph. The studies indicate that ethanolic extract of *A. nervosa* whole plant producing promising results in treatment of nephrotoxicity.

**Key words:** Alkaloids, *Argyeria nervosa*, flavonoids, gentamicin, steroids

**INTRODUCTION**

Medicinal herbs play an important role in the development of potent therapeutic agents. About 80% of people in developing countries depend on traditional medicine based largely on species of plants and animals for primary health care[1] Herbal drugs are currently in demand and their popularity is increasing day by day. In indigenous systems of medicine, about 800 plants have been used and around 500 plants with medicinal use are mentioned in ancient literature. Herbal drugs refer to plant materials or herbals, which involves the whole use of plants or parts of plants, to treat diseases. These drugs are oldest form of health

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care known to mankind. World Health Organization has set guidelines for evaluation of safety, efficacy, and quality of herbal drugs.\[2\] Herbal drugs are the chief constituents of traditional medicine and common source in Ayurvedic, homeopathic, naturopathic and other medicine systems. The use of herbal drugs due to toxicity and side effects of allopathic medicines has lead to rapid growth in the number of herbal drug manufacturers.\[3\] For the past few decades, herbal drugs have been more consumed by the people with no prescription. Some drugs have been discontinued due to their toxicity, while others combined with additional herbs to counter balance side effects.\[4\] Toxicity from medicinal plants has been identified and it usually may be due to wrong identification of plants in the form in which they are sold or incorrect preparation and administration by improper trained personnel. There are some drugs like plants remedies that their actions approach that of pharmaceuticals.\[5\]

**Advantages of Herbal Drugs**\[6\]
- High low/minimum cost
- Complete accessibility
- Enhanced tolerance
- More protection
- Fewer side effects
- Potency and efficiency is very high.

**Disadvantages of Herbal Drugs**\[7\]
- Not able to cure rapid sickness and accidents
- Risk with self-dosing
- Complexity in standardizations.

Nephrotoxicity is one of the most common kidney problems and occurs when your body is exposed to a drug or toxin that causes damage to your kidneys. When kidney damage occurs, you are unable to rid your body of excess urine, and wastes.\[8\] Your blood electrolytes will all become elevated. Nephrotoxicity is also a condition when kidneys become unable to filter waste products from blood, lose their filtering ability, accumulation of dangerous waste. It consists of whole disorders results to damage of different nephron segments.\[9\] Toxicity of nephron produced by drug includes both glomerular and tubular injuries leading to acute or chronic functional changes.\[10\]

**Statistics**

Nephrotoxicity is recognized as major medical problem worldwide. The global burden of disease (GBD) study according to 2015 ranked chronic kidney disease 17th among the causes of deaths globally. In United States, the chronic kidney disease increased from 12% to 14% between 1988 and 1994 and from 1999 to 2004 it remained relatively stable since 2004 [Figure 2].\[11\] Women’s (15.93%) have more prevalence than men (13.52%). African Americans (17.0%) and Mexican Americans (15.29%) are more likely to have chronic kidney disease than Caucasians (13.99%). In Australia, in the year 2013, there were 5100 new cases of end stage kidney disease (ESKD).\[12\] The ESKD incidence rate was 1.5 times as high for males as for females. The rate of ESKD in 2013 was highest for those aged 75 and over. This rate was 7 times as high as the rate for those aged 55–64 (26 per 10,000).\[13\] In India, GBD 2015 ranks nephrotoxicity as the eighth leading cause of death. Deaths due to renal toxicity constituted 2.9% of all deaths in 2010–2013 among 15–69 years old, an increase of 50% from 2001–2003. The reported proportion of renal toxicity death is close to GBD 2015 estimate of 3.04% up from 1.94% in 2000 [Figure 3].\[14\]

**Symptoms**

Many different symptoms can occur during nephrotoxicity. Possible symptoms include:

- A reduced amount of urine
- Swelling of legs, ankles and feet
- Shortness of breathe
- Drowsiness or fatigue

![Figure 1: Tannins present](image1.jpg)

![Figure 2: Glycosides present](image2.jpg)
Complications

India has rich heritage of medicine herbs which are used by the local population and traditional practitioners for the treatment of several conditions. Plants and other natural substances have been used as the rich source of medicine [Figure 4]. All ancient civilizations have documented medicinal uses of plant in their own ethno botanical texts. The list of drugs obtained from plant source is fairly extensive. All the above data acquired by extensive literature survey and folk medicine practice inspired to go for the possible nephron protective activity of *Argyreia nervosa* [Figure 5]. Due to its potent pharmacological activities, low toxicity and economic viability *A. nervosa* is well known plant traditionally, it has been used as antibacterial, antifungal, anti-analgesic, anti-inflammatory, anti-oxidant, anti-pyretic, aphrodisiac, cardio protective, hypoglycaemic, no tropic, and nephron protective. But nephro protective activity is not explored scientifically so the present study is to evaluate the nephro protective activity of *A. nervosa* whole plant in albino Wister rats.[17]

MATERIALS AND METHODS

Drugs and Chemicals

Picric acid, alkaline buffer reagent, standard creatinine, surfactant, urea reagent, acid reagent, diaceylmonoxime (DAM) reagent, urea standard, buffer reagent, enzyme reagent, uric acid standard, *A. nervosa*, gentamicin, di-ethyl ether [Figure 6].

Plant Material and Authentication

*A. nervosa* whole plant powder was procured from local market and authenticated at Acharya Nagarjuna University, Guntur, Andhra Pradesh.

Figure 3: Carbohydrates present

Figure 4: Serum creatinine levels (mg/dl)

Figure 5: Alkaloids present

Figure 6: Steroids present
Experimental Animals

In this work adult Wistar albino rats of either sex, weighing 150–200 g were purchased from the Sainath agencies, Basheerabadh, Hyderabad-48. The animals were housed in polypropylene cages under hygienic condition at room temperature 25 ± 4°C with relative humidity of 50–60%, on 12 h. Light/12 h. Dark cycle with standard rodent diet and water ad libitum, they were allowed to acclimatize to the conditions in the animal house of A. M. Reddy Memorial College of pharmacy.[19]

Plant Extraction

The whole plant was shade dried and powdered by using mechanical grinder. The powder of the plant soxhlated with 90% ethanol for 48 h by maintaining temperature that not exceeding the boiling point of the solvent and the extract was separated from the solvent by using filtration, for the elimination of ethanol the extract was subjected to vacuum and the extract was stored in sterile bottle at refrigeration condition. The extract was subjected to preliminary phytochemical studies.[20]

Phytochemical Studies

The phytochemical studies are carried out on the extract for analysing the presence of flavonoids, alkaloids, tannins, carbohydrates, cardiac glycosides, anthrax Quinone glycosides, amino acids, phenolsetc.[21]

Tests for Flavanoids

1. Shinoda test
   Sample + 5 ml of 95% of ethanol + few drops of Conc. HCL + 0.5 g of magnesium turnings → pink colour is observed.

2. Ferric chloride test
   Sample (alcoholic) + few drops of neutral ferric chloride solution → green colour is observed.

3. Lead acetate test
   Sample (alcoholic) + few drops of 10% lead acetate → yellowish precipitate is observed.

Tests for Steroids

1. Salkoswski test
   Sample + chloroform solution + Conc. H2SO4 → red colour is observed.

2. Liebermann burchards test
   Sample + chloroform solution + few drops of acetic anhydride + 1 ml of Conc. H2SO4 → reddish ring at the junction of two layers.

Tests for Alkaloids

Sample + ammonia + chloroform solution + dil. HCL (sample used for alkaloidal test).

1. Mayer’s test
   Sample + potassium mercuric iodide + few drops of Mayer’s reagent creamy white precipitate.

2. Wagner test
   Sample + solution of iodine in potassium iodide + few drops of Wagner’s reagent reddish brown precipitate are obtained.

3. Hager’s test
   Sample + saturated solution of picric acid + Hager’s reagent → yellowish precipitate is observed.

4. Dragendorff’s test
   Sample + solution of potassium bismuth iodide + few drops of Dragendorff’s reagent → reddish brown precipitate is observed.[22]

Tests for Tannins

1. Ferric chloride test
   Sample + 1% of ferric chloride solution → blue, green (or) brownish green colour is observed.

2. Gelatine test
   Sample + few drops of 1% of solution of gelatine containing 10% of sodium chloride → white precipitate.

Tests for Carbohydrates

1. Molisch’s test
   2 ml Sample + add 2 drops of alpha napthol solution + few drops of conc. H2SO4 (1 ml) → a deep violet coloration is produced at junction of two layers.

2. Iodine test
   2 ml of sample + 2 drops of iodine solution a blue-black colour is observed.

3. Fehling’s test
   2 ml of sample + few drops of Fehling’s - A and Fehling’s - B solutions and it is boiled for 10 min → yellow (or) brownish red precipitate is observed.

4. Barfoed’s test
   2 ml of sample + 2 ml freshly prepared Barfoed’s reagent and it is boiled for 3 min later it is cooled. A deep blue colour is formed with a red precipitate settled at bottom or sides of test tube were observed.

5. Seliwanoff’s test
   1 ml sample + 3 ml of Seliwanoff’s reagent and boil in water bath for 2 min → a cherry red colored precipitate (or) a faint red colour is observed [Figure 7 and Table 1].[23]
6. Bial’s test
0.2 ml sample + 3 ml of Bial’s reagent and heat the solution in water bath for 2 min → a blue-green product (or) a muddy brown to grey product is observed.

7. Benedict’s test
1 ml of sample + 5 ml of benedict’s solution and heat the solution for 3 min and allow them to cool → a deep blue colour is formed with a red precipitate at the sides of the test tube.

8. Osazone test
4 ml of sample + 3 ml of phenyl hydrazine hydrochloride solution and boil the solution for 30 min + cool the solution and observe the crystals under microscope → formation of beautiful yellow crystals of osazone needle shaped crystals (or) hedgehog crystals (or) sunflower shaped crystals.

**Tests for Cardiac Glycosides**

1. Raymond’s test
Sample + few ml of 50% ethanol and few 0.1 ml of 1% solution of m-dinitrobenzene in ethanol + 2–3 drops of 20% sodium hydroxide solution → violet colour appears [Figure 8].

2. Legal test
Sample + few ml of pyridine + 2 drops of nitro prusside + a drop of 20% sodium hydroxide solution a deep red colour is produced.

3. Killer-killianis test
Sample + mixture of 1% ferric sulphate solution in 5% glacial acetic acid + 1 (or) 2 drops of conc. sulphuric acid → a blue colour is observed.

4. Xanthydrol test
Sample is heated with 0.1–5% solution of xanthydrol in glacial acetic acid containing 1% hydrochloric acid a red colour is observed.

5. Baljet test
Take a piece of lamina (or) thick section of leaf + sodium picrate reagent yellow to orange colour is observed.

6. Kedde test
Sample + small amount of kedde reagent (mix equal volumes of 2% solution of 3, 5 dinitrobenzoic acid in methanol and a 7.5% aqueous solution of KOH) → a blue or violet colour observed that faded out in 1–2 h.

7. Antimony trichloride test
Sample + solution of antimony trichloride + trichloroacetic acid → heat the mixture blue (or) violet colour is observed.

**Tests for Anthraquinones Glycosides**

1. Borntranger’s test
Powdered form of sample + diluted with sulphuric acid + filtered and cooled + chloroform (or) benzene + dilute ammonia → ammonical layer becomes pink to red.

2. Modifiedanthraquionones test
0.1 g of sample + 5 ml of 5% solution of ferric chloride + 5 ml of dilute hydrochloric acid + heat for 5 min + cool the solution + shake the solution with benzene + separate the layer and add an equal volume of dilute ammonia a pinkish red colour is observed [Figure 9].

**Tests for Amino Acids**

3. Ninhydrin test
1 ml of sample + few drops of ninhydrin reagent + boils the solution for 5 min and later cool the solution.

4. Xanthoproteic acid test
1 ml of sample + few drops of nitric acid + boil the solution for few minutes + cool the solution a.

5. Pauly’sdiazo test
Sample + 1 ml of sulphanillic acid reagent + few drops of prechilled sodium nitrite solution + few drops of prechilled amino acid → addition of sodium carbonate until colour disappears.
Test for Phenols

1. Ferric chloride test
   Sample + mixture of ethanol and water + dilute ferric chloride → blue (or) green (or) purple (or) red.

2. Bromine water test
   Sample (phenolic) + few ml of bromine water → white precipitate are observed.

3. Lieberman’s test
   Sample + few drops of sodium nitrate + heat the mixture and cool it few drops of conc. sulphuric acid → blue colour is observed + treated with water → red colour is observed + few drops of strong alkali solution → blue (or) green colour is observed.[27]

Acute Oral Toxicity Studies

The test dose 200 mg/kg and 400 mg/kg was chosen as experimental doses according to below references.

Experimental Design

The animal experiments were performed after acclimatization animals were separated into four groups of each group with 6 animals was placed in separate cages.[28]

Methodology

- Group I: After acclimatization of animals, the vehicle was administered orally for 8 days of experimental period.
- Group II: Animals were treated with gentamicin with a dose of 80 mg/kg/day for 8 days intraperitoneal.
- Group III: Animals were treated with gentamicin with a dose of 80 mg/kg intraperitoneal and extract of *A. nervosa* 200 mg/kg per day orally for 8 days.
- Group IV: Animals were administrated with gentamicin with a dose of 80 mg/kg intraperitoneal and extract of *A. nervosa* 400 mg/kg orally per day for 8 days.

After experimental period some biochemical parameters were estimated to evaluate the nephro activity.

Collection of Blood

The rats were anesthetised with diethyl ether after 24 h from last injection and the blood samples were collected from retro orbital [Figure 10].[29]

Estimation of Serum Creatinine

To estimate the creatinine levels a PFF of plasma or serum with an alkaline picrate solution giving rise to a red colour (alkalinecreatinine picrate) due to the reaction, which is measured at 520 nms, at 37°C.[30]

Estimation of Uric Acid

Uric acid is estimated by Uri case/Phenol-Amino-Phenazone method. In this method Uri case converts uric acid to allantoin and hydrogen peroxide. The hydrogen peroxide formed further reacts with a phenolic compound and 4-aminoantipyrine by the catalytic action of peroxidase forms a red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of uric acid present in the sample.[31]

Estimation of Urea

Urea levels are estimated by DAM method. In this method urea in an acidic medium condenses with diacetylimonoxide at 100°C forms a red coloured complex. Intensity of the colour formed is directly proportional to the amount of urea present in the sample [Figure 11].[32]
RESULTS

Percentage Yield of EEAG

The shaded parts of *A. nervosa*. Weighing about 200 g was extracted by Soxlet extraction method using 90% ethanol. The weight of ethanolic extract obtained was 12 g. Its percentage yield is calculated by following formula:

\[
\text{Percentage yield} = \frac{\text{Weight of extract obtained}}{\text{Weight of crude powder}} \times 100
\]

Preliminary Phytochemical Investigation

The results of the preliminary phytochemical screening of the ethanolic extract of whole plant of *A. nervosa* were shown in the Table 2 the ethanolic extract gave positive results for flavonoid, alkaloids, steroids, tannins, carbohydrates, glycosides, phenols.

Effect of EEAG on Serum Biochemical Parameters

A significant version of elevated levels of serum urea, uric acid and creatinine in groups treated with EEAG as compared to disease control group was tabulated in Table 3 and their graphical representation was showed in figures respectively.

DISCUSSION

Gentamicin belongs to a class of aminoglycoside antibiotic. It is used in the form of injection to treat bacterial infection. Over dosing and frequent administration of gentamicin causes side effects associated with neurotoxicity, ototoxicity, kidney damage, swelling, itching, stomach upset. Ultimately gentamicin having a considerable effect on renal functions. (Aminoglycosides in gentamicin which have to be filtered they are incorporated into lysosomes after first interacting with phospholipids on brush border membrane their accumulation causes toxic effect within the tubular cells by altering the phospholipid metabolism and their direct effect on cells also causes renal vasoconstriction). Acute renal failure caused by gentamicin is due to oxidative stress generated by free radicals. The reactive metabolites generated during the intracellular metabolism of drugs are toxic for cell as are free radicals. This cellular injury and necrosis can be caused through several mechanisms including DNA damage, peroxidation of membrane lipids, protein denaturation, results in considerable modification of structure and function. In the present study renal damage was produced upon induction of gentamicin at a dose of 80 mg/kg. The significant renal damage when compared to control group was revealed by increase in serum creatinine, serum urea and serum uric acid levels. By administration of EEAG a predominant decrease in renal disfunction was observed when compared to gentamicin alone induced group which was demonstrated by changes in biochemical parameters. Anti-oxidants have

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**Table 1: Experimental design EEAN**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Number of animals (rats)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vehicle control</td>
<td>6</td>
</tr>
<tr>
<td>Group 2</td>
<td>Gentamicin (80 mg/kg)</td>
<td>6</td>
</tr>
<tr>
<td>Group 3</td>
<td>Gentamicin (80 mg/kg) + EEAN (200 mg/kg)</td>
<td>6</td>
</tr>
<tr>
<td>Group 4</td>
<td>Gentamicin (80 mg/kg) + EEAN (400 mg/kg)</td>
<td>6</td>
</tr>
</tbody>
</table>

EEAN: Ethanol extract of *A. nervosa*, *A. nervosa*: *Argyreia nervosa*

**Table 2: Phytochemical test results**

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid’s</td>
<td>Present</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Present</td>
</tr>
<tr>
<td>Steroids</td>
<td>Present</td>
</tr>
<tr>
<td>Tannins</td>
<td>Present</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Present</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Present</td>
</tr>
<tr>
<td>Phenols</td>
<td>Present</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Table 3: Test parameters**

<table>
<thead>
<tr>
<th>Groups and treatment</th>
<th>Parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>Normal control</td>
<td>0.58</td>
</tr>
<tr>
<td>Gentamicin (80 mg/kg)</td>
<td>1.71</td>
</tr>
<tr>
<td><em>A. nervosa</em> (200 mg/kg)</td>
<td>1.55</td>
</tr>
<tr>
<td><em>A. nervosa</em> (400 mg/kg)</td>
<td>0.92</td>
</tr>
</tbody>
</table>

*A. nervosa*: *Argyreia nervosa*
the ability to protect the body from the damage caused by the free radicals by stabilising or deactivating the free radicals which induces oxidative stress. To treat gentamicin induced nephrotoxicity several phytochemical derivatives having anti-oxidant property were used experimentally. From the phytochemical analysis of EEAG reveals the presence of flavonoids, tannins, alkaloids, glycosides, phenols which are important for anti-oxidant activity to act as free radical scavenger. Thus the present study proves the free radical scavenging activity by anti-oxidant property of ethanolic extract of *A. nervosa*.

In this experiment the nephrotoxicity was induced by gentamicin at a dose of 80 mg/kg for 8 days. It was demonstrated by increase in serum creatinine (1.71 mg/dl), serum uric acid (9.03 mg/dl), serum urea (66.3 mg/dl) from 0.58 mg/dl, 3.23 mg/dl, 33 mg/dl of control group. The nephroprotective activity of *A. nervosa* (200 mg/kg) along with gentamicin was revealed by decrease in the serum creatinine 1.55 mg/dl, serum uric acid 4.63 mg/dl, serum urea 63 mg/dl. Upon administration of increased dose of v 400 mg/kg shows the decrease in the nephrotoxic effect of gentamicin testified by the predominant decrease in serum creatinine (0.92 mg/dl), serum uric acid 2.86 mg/dl, serum urea 56.6 mg/dl by the end of experiment certain biochemical parameters levels related to kidney function were reduced in the treatment groups from the toxic effects of gentamicin. Hence from experiment higher dose of EEAG 400 mg/kg has significant nephroprotective activity and from the previous investigation it is also proved that *A. nervosa* having the anti-fungal, antibacterial, anti-oxidant, anti-inflammatory, anti-tumour, antipyretic, aphrodisiac, cardioprotectivity, hypoglycaemic activity, no tropic activity, analgesic activity. Thus the findings of the present study validate the nephroprotective activity of *A. nervosa* for the management of renal failure caused by the gentamicin induced nephrotoxicity. Upon further investigations on this plant having a considerable impact on developing the ideas on nephroprotective activity.

**CONCLUSION**

The present study was an attempt to evaluate ethanolic extract of *A. nervosa* whole plant for nephroprotective activity. The plant whole plant was screened for biochemical parameters (serum creatinine, serum uric acid, serum urea). The ethanolic extract has produced effective nephroprotective action as evident by results obtained from the studies. The interpretation of the results was done after subjecting the data obtained from various studies by simple graph. The studies indicate that ethanolic extract of *A. nervosa* whole plant producing promising results in treatment of nephrotoxicity.

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