

Reno productive activity of *Ipomoea digitata* in gentamicin induced kidney dysfunction

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Keywords	Abstract
Nephrons Gentamicin Ureters <i>Ipomoea digitata</i> Kidney dysfunction Nephroprotective activity	Kidneys endowed with million units termed as “nephrons” that act as natural sieves. The kidneys provide the final common pathway for the excretion of many drugs and their metabolites and therefore are frequently subjected to high concentrations of potentially toxic substances. Administration of several antibiotics like <i>Gentamicin</i> causes kidney dysfunction. Rats treated with <i>Gentamicin</i> developed significant kidney dysfunction was observed from increased level of urea, creatinine, sodium and decreased level of protein, potassium and non enzymatic antioxidants such as <i>vitamin C</i> and <i>vitamin E</i> . The plant, <i>Ipomoea digitata</i> is found to have nephroprotective activity.

1. Introduction

The kidneys are sophisticated reprocessing machines. Every day kidneys process about quarts of blood to sift out about 2 quarts of waste products and extra water. The waste and extra water become urine, which flows to bladder through tubes called ureters. The waste in blood become from the normal breakdown of active tissues from the food. Body uses the food for energy and self repair. If kidneys did not remove these wastes, the wastes would build up in the blood and damage the body. The actual filtering occurs in tiny units inside your kidneys called nephrons. Every kidney has about a million nephrons, in the nephrons, a glomerulus which is a tiny urine collecting tube called as tubule. A complicated chemical exchange takes place, as waste material and water leave blood and enter urinary system. At first, the tubules receive a combination of waste materials and chemicals that your body can still use. Kidneys measure out chemicals like sodium, phosphorus, and potassium and release them back to the blood to return to the body. In this way, kidneys regulate the body's level of these substances. The right balance is necessary for life, but excess levels can be harmful (National Institutes of Health, 2005).

2. Materials and Methods

Animals

Male albino rats of wistar strain approximately weighing 100-120 gm were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. 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 ± 2 °C) and 12 h light/dark cycle) throughout the experimental period. All the animals were fed with stander pellet diet and water were provided adlibitum. They were acclimatized to the environment for one week period to experimental use. The animal feed composition is crude Protein (22.3%), Crude Oil (4.07%), Crude fiber (4.02%), Ash (8.02%) and Sand Silica (1.02%).

Chemicals

Thiobarbituric acid (TBA), 2,4 Dinitro phenyl hydrazine (DNPH), Red-VAD Glutathione (GSH) Gentamicin were purchased from Sigma Chemical Company, Mumbai. All other chemicals and reagents used in this study was analytical grade with high purity and were obtained from Glaxo Laboratories, and Sisco Research Laboratories, Mumbai, India.

Plant material and preparation of extract

The root of *Ipomoea digitata* was collected from Thanjavur, Tamil Nadu. The collected roots were cut into small pieces and shade dried at room temperature. The root was soaked with ethanol (50%) for 48 h. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used. The root extract was dissolved in distilled water just before oral administration.

Induction of nephrotoxicity

Experimental nephrotoxicity was induced by intra peritoneal injection of gentamicin at a dose of 50 mg/kg body weight.

Experimental Design

Body weight of animal were recorded and they were divided into three groups of six animals each as follows.

Group I : Normal animals received with standard fed and water to allow ad libitum.

Group II: Animals received daily intra peritoneal injection of gentamicin (80mg/kg b.wt) for five days, this dose has already has been shown to produce nephrotoxicity (Abdel et al., 1994)

Group III: Animally received gentamicin as group II. After 24 hours of last injection of gentamicin treatment was started at a dose of 500 mg/kg/b.wt. of root of *Ipomoea digitata* for 5 days.

Collection of blood and preparation of Serum sample:

At the end of the experimental period, the animals were anaesthetized using chloroform vapors prior to dissection. Blood was collected by Cardiac puncture into serum separator tubes. The blood was allowed to clot by standing at room temperature for another 30 minutes. The result was allowed to Clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minutes. The resultant clear part was centrifuged at 3000 rpm for 10 min, then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

Tissue homogenate:

Immediately after blood collection, the animals were sacrificed by cervical dislocation and the kidney was dissected out, washed with ice-cold physiological saline, and a homogenate was prepared in 0.1 Tris HCL buffer (PH 7.4). The homogenate was centrifuged and the supernatant was used for the assay of various biochemical parameters.

3. Results

The present study was carried out to evaluate the Reno protective activity of *ipomoea digitata* in gentamicin induced kidney dysfunction. The observations made on different groups of experimental and control animals were compared as follows.

Table I represents the levels of MDA and GSH in serum and kidney of normal and experimental rats.

Group II gentamicin intoxicated rats showed a significant increased in the level of MDA in serum and kidney when compared to Group I rats. Group III gentamicin intoxicated rats treated with *ipomoea digitata* significantly decreased in the level of MDA in serum and kidney compared to group II.

Group II gentamicin intoxicated rats showed a significant decreased in the level of GSH in serum and kidney when compared to Group I rats. Group III gentamicin intoxicated rats treated with *ipomoea digitata* significantly increased in the level of GSH in serum and kidney as compared to group II.

Table I. Effect of *ipomoea digitata* on MDA and GSH in serum and kidney in experimental rats.

Parameters	GROUP I (NORMAL)	Group II (Gentamicin)	Group III (<i>Ipomoea digitata</i>)
Serum			
MDA	12.0 ± 1.45	21.91 ± 1.77	8.58 ± 1.46*
GSH	50.95 ± 7.41	31.42 ± 9.58	42.61 ± 5.47*
Kidney			
MDA	4.75 ± 0.04	9.58 ± 0.06	4.75 ± 0.04*
GSH	62.13 ± 1.09	42.13 ± 2.66	62.61 ± 1.11*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II (P<0.001).

Table II represents the levels of vitamin C and vitamin E in kidney of normal and experimental rats.

Group II gentamicin intoxicated rats showed a significant decreased in the level of vitamin C when

compared to group I rats. Group III gentamicin intoxicated rats treated with *ipomoea digitata* significantly increased in the level of vitamin E in kidney as compared to group II.

Table II. Effect of ipomoea digitata on vitamin C and vitamin E in kidney of experimental.

Parameters	Group I (Normal)	Group II (Gentamicin)	Group III (Ipomoea digitata)
Vit. C (mg /gt)	42.88± 2.058	37.12 ± 2.018	43.13±1.073*
Vit. E (mg /gt)	0.90 ± 0.064	0.39 ± 0.056	0.91± 0.03*

Values were expressed as mean ± SD for six rats in each group.

Significantly different from Group II (P<0.01).

Table III represents the levels of urea and creatinine in serum of normal and experimental rats.

Group II gentamicin intoxicated rats showed a significant increased in the level of urea when compared to Group I rats. Group III gentamicin intoxicated rats treated with ipomoea digitata significantly decreased in the level of urea when compared to group II.

Group II gentamicin intoxicated rats showed a significantly increased in the level of creatinine when compared to Group I rats. Group III gentamicin intoxicated rats showed a significantly decreased in the level of creatinine as compared to group II.

Table III. Effect of ipomoea digitata on urea and creatinine in plasma serum experimental rats.

Parameters	Group I (Normal)	Group II (Gentamicin)	Group III (Ipomoea digitata)
Urea (mg / dl)	35.0 ± 0.55	71.66 ± 0.54	35.0 ± 0.59*
Creatinine (mg / dl)	0.69 ± 0.049	1.22 ± 0.23	0.63 ± 0.06*

Values were expressed as mean ± SD for six rats in each Group.

* Significantly different from Group II (P<0.01)

Table IV represents the levels of protein in kidney and serum albumin of normal and experimental rats.

Group II gentamicin intoxicated rats showed a significantly decreased in the level of protein and

serum albumin when compared to group I rats. Group III gentamicin intoxicated rats treated with ipomoea digitata significantly increased in the level of protein and serum albumin when compared to group II.

Table IV. Effect of ipomoea digitata on protein in kidney and serum albumin experimental rats.

Parameters	Group I (Normal)	Group II (Gentamicin)	Group III (Ipomoea digitata)
Protein (mg / gt)	17.14 ± 2.13	14.18 ± 1.44	18.07 ± 2.92 *
Albumin (mg / dl)	4.38 ± 0.878	3.42 ± 0.09	4.26 ± 0.556*

Values were expressed as mean ± SD for six rats in each Group.

* Significantly different from Group II (P<0.001)

Table V represents the levels of sodium and potassium in serum of normal and experimental rats.

Group II gentamicin intoxicated rats showed a significantly increased in the level of sodium when compared to Group I rats. Group III gentamicin intoxicated rats treated with ipomoea digitata

significantly decreased in the level of sodium when compared to group II.

Group II gentamicin intoxicated rats showed a significantly decreased in the level of potassium when compared to Group I rats. Group III gentamicin intoxicated rats treated with ipomoea digitata significantly increased in the level of potassium and compared to group II.

Table V. Effect of ipomoea digitata and sodium and potassium in serum experimental rats.

Parameters	Group I (Normal)	(Group II (Gentamicin)	Group III (Ipomoea digitata)
Sodium (meq / dl)	118.67 ± 6.01	174.09 ± 5.32	133.42 ± 4.76*
Potassium (meq / dl)	4.41 ± 0.36	3.41 ± 0.67	3.83 ± 0.66*

Values were expressed as mean ± SD for six rats in each group.

* Significantly different from Group II. (P<0.01; P<0.01)

4. Discussion

Kidneys endowed with million units are termed as nephrons that act as natural sieves. Unfortunately, kidney diseases may be silent for long time. Early detection is the key to preventing kidney diseases, thereby significantly reducing the associated morbidity and mortality. The kidneys provide the final common pathway for the excretion of many drugs and their metabolites and therefore are frequently subjected to high concentrations of potentially toxic substances (Schetz et al., 2005).

Drugs and their metabolites are taken up selectively and concentrated into the urine, so high intracellular concentrations are attained, particularly in the renal medulla which has relatively little vasculature compared with the cortex. As a result, direct toxic damage occurs, generally affecting the renal tubular cells and renal papillae. Nephrotoxicity of this type tends to be dose dependent. Many groups of drugs can cause renal damage including allopurinol, carbamazepine, cimetidine, erythromycin, gentamicin, (Karakoyun et al., 2009). In recent years medicinal plants are used to prevent nephrotoxicity in both animal model and human subjects. Therefore in the present study, evaluated the nephroprotective activity of Ipomoea digitata on gentamicin induced rats.

Gentamicin (GM) is widely used amino glycoside antibiotic, is recognized as possessing significant nephrotoxic potential in man and experimental animals (Humes and Weinberg, 1986). There is an accumulating body of evidence supporting the concept that reactive oxygen metabolites including free radicals participate in renal tissue injury (Ray et al., 1996). GM was reported to enhance the generation of hydrogen peroxide and oxygen free radicals (Rehan et al., 1984). Reactive oxygen species may produce cellular injury and necrosis via, several mechanisms including per oxidation of membrane lipids, protein, denaturation and DNA damage (Cuzzocrea et al., 2002). In this context a marked increase in the concentration of serum and kidney MDA were observed in gentamicin induced rats when compared to control rats. Administration of Ipomoea digitata significantly decreased the levels of MDA in gentamicin induced rats.

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 diseases states including cancer, neurodegenerative 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 (Ballatori et al., 2008). In the present study, declined level of serum and kidney GSH were observed in gentamicin induced rats when compared to control rats. The decrease in GSH level represents increased utilization for neutralizing free radicals generated from gentamicin. Supplementation of Ipomoea digitata to gentamicin induced rats, attained near normal level. Our results are compatible with the study of (Silan et al., 2007).

An antioxidant has been defined as “any substance that, when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. When ROS /RNS are generated in view, their actions are opposed by intricate and coordinated antioxidant lines of defense systems. This includes enzymatic and non enzymatic antioxidant that keeps in check ROS / RNS level and repair oxidative cellular damage (Halliwell and Gutteridge, 1999). Circulatory non enzymatic antioxidant such as vitamin E and vitamin C are free radical scavengers. Their synergistic action in scavenging oxygen derived free radicals is well documented (Rock et al., 1996). Vitamin E reacts with lipid peroxy radicals acting as a chain terminator of lipid per oxidation while vitamin C helps to maintain the level of vitamin E at optimum concentrations. The levels of Kidney vitamin E and vitamin C in the present study were significantly reduced in gentamicin treated rats than in the experimental control rats. Supplementation with Ipomoea digitata to gentamicin treated rats resulted in near normal levels of vitamin E and vitamin C.

Urea is an end product of protein catabolism. It is freely filtered by the glomerulus, passively reabsorbed in the 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 (Stevens and Levey, 2005). Drugs that can increase urea levels include allopurinol, some diuretics, gentamicin and indometacin (Rodnan et al., 1975). In the present study also observed the increased level of urea in gentamicin intoxicated rats. Supplementation of *Ipomoea digitata* restored the increased level of urea in gentamicin induced rats. (Lokesh and Amit, 2006).

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) (Goulding et al., 2009). 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 (Stevens and Levey 2005). This results supports our findings. Administration of *Ipomoea digitata* restored the level of creatinine in gentamicine treated rats (Saravanan and Nalini 2007).

Acid, bases and salts are collectively called electrolytes. Electrolyte imbalance can leads 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 kidneys function (Springate et al., 1992). In the present study, gentamicin treated rats significantly lower in potassium levels and higher in sodium levels when compared with normal control rats. This due to antiport transport system of sodium and potassium i.e. the increased excretion of potassium is promoted the reabsorption of sodium. Administration of *Ipomoea digitata* restored the normal level of sodium and potassium in gentamicin treated rats. This result consisted with Silan et al (2007) studies.

5. Summary and Conclusion

The normal function of the kidney is vital to the existence of human life because it is the main organ that eliminates waste products of metabolism and regulated various ionic balances in the fluids of the body. These paramount functions of the kidney are affected in a little way or grossly, depending upon the severity of the kidney failure. Thus, the

protection or preservation of kidney function is essential for maintenance of healthy life.

Many medical and scientific researchers are convinced that uncontrolled free radical activity in the body is directly associated with a number of health problems. Free radical reactions have been implicated in the pathology of may human diseases including atherosclerosis, ischemic, heart and kidney disease, the aging process, inflammation, diabetes and other disease conditions.

Gentamicin (aminoglycoside) antibiotics are widely used as antibacterial agents for the treatment of severe aerobic gram (-) infections. However, amino glycoside induced nephrotoxicity is limiting factors for their clinical use and oxygen free radicals have been involved in amino glycoside induced nephrotocicity. As plant produce a lot of antioxidants to control the oxidative stress, they can represent a source of new compounds with antioxidant activity. A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models.

Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life several plant products are known to exhibit creditable medicinal properties for the treatment of various ailments and need to be explored to identify their potential application in prevention and therapy of human ailments. The medicinal value of the chosen plant *Ipomoea digitata*

In the present study was evaluated the nephro protective activity of *Ipomoea digitata* Gentanmicin, an amino glycosides was chosen to induce nephrotoxicity.

Rats treated with gentamicin developed significant kidney dysfunction was observed from increased level of urea, creatinine, sodium and decreased level of protein, potassium and non enzymatic antioxidants such as vitamin C and vitamin E. Oxidative stress markers such as MDA and GSH were also altered in this study. Supplementation of *Ipomoea digitata* gentamicin intoxicated rats restored the altered above said parameters. The nephro protective activity of *Ipomoea digitata* may be due to the phytochemical constituents such as flavonoids, alkaloid, saponin etc. present in it. Some of these compounds have potential nephro protective activity. Future studies required which compound is responsible for the nephroprotective activity.

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