Nephroprotective Activity of Aristolochia indica Leaf Extract Against Gentamicin Induced Renal Dysfunction

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Abstract
Nephrotoxicity is one of the important side effects of Gentamicin antibiotics. The aim of the study was to determine the protective effect of Aristolochia indica leaf extract on Gentamicin induced nephrotoxicity in rats using biochemical approaches. Oxidative stress is the main factor in Gentamicin (GM) induced nephrotoxicity. Wistar rats divided into three groups, the first group served as normal. The second group received Gentamicin (40 mg/kg, 2 weeks/day) along. Third group received Gentamicin (40 mg/kg, 2 weeks/day) and co-treatment with Aristolochia indica leaves (500 mg/kg) extract for 2 weeks. Nephrotoxicity was assessed by measuring the abnormal levels of serum creatinine, urea and sodium and decreased level of protein and potassium. The antioxidant defence enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) of kidney tissue were also measured at the end of the treatment schedule. Treatment with Aristolochia indica leaves (500 mg/kg) significantly (p < 0.05) restored the levels of serum creatinine, urea, sodium, protein and potassium. Significantly (p < 0.05) increased the antioxidant defence enzyme levels of SOD, GPx and CAT on treatment with Aristolochia indica. The results suggest that an Aristolochia indica leaf has the potential in preventing the nephrotoxicity induced by Gentamicin.

Key words: Gentamicin, Aristolochia indica, Oxidative stress, antioxidant defence enzymes

INTRODUCTION
The kidneys are sophisticated reprocessing machines. Every day; kidneys process about 200 quarts of blood to shift 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 wastes in blood come from the normal breakdown of active tissues and from the food. Body uses the food for energy and self-repairs. Body has taken its needs from the food. Waste is sent to the blood. If kidneys did not remove these wastes, the wastes would build up in the blood and damage the body (Iwara et al., 2012). 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. Drugs and their metabolites are taken up 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 (Tavafi et al., 2012).

Drug induced renal failure is a well-recognized phenomenon, although the incidence of drug – induced renal disease remains uncertain. However, some reports suggest that between 5 - 20 percent of cases of acute renal failure can be directly attributed to drugs and chemicals, although minor damage may pass undetected. It is important to be aware of the types of drug that can induce, renal impairment because, if suspected and acted on early, the damage to the kidney may be reversible. Many drugs have been reported to cause acute interstitial nephritis, including allopurinol, carbamazepine, cimetidine, erythromycin, gentamicin, interferon, aminocycline, penicillin, phenytoin, propanolol, sulphonamids, azathioprine, cephalosporin, furosemide, isoniazid, NSAIDs, phenobarbitone, phenindione, rifampicin and thiazides (Mohana Lakshmi et al., 2012).

Gentamicin is an amino glycoside antibiotic that is still commonly used in the treatment of life-threatening infections. The broad-spectrum activity against aerobic gram positive and gram negative organisms, their chemical stability, and their rapid bactericidal action has often made them first-line drugs in a variety of clinical situations.
However, higher concentrations of these antibiotics are nephrotoxic. In some cases, it may give serious side effects are so severe that the use of the drug must be discontinued. It has been estimated that up to 30% of patients treated with aminoglycosides for more than 7 days show some signs of nephrotoxicity (Saumya et al., 2011).

Medicinal plants are a major source of biodynamic compounds of therapeutic values. These are assuming greater importance in the primary health care of individuals and communities in many developing countries. There has been an increased demand in international trade because of very effective, cheaply available, less or no side effects and used as alternative to allopathic medicines. Medicinal plants are believed to be much safer and used in the treatment of various ailments (Singhet et al., 2008). Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world. Indeed along with the dietary measures, plant preparation formed the basis of the treatment of the disease until the introduction of allopathic medicine. Medicinal plants, since times immemorial have been used in virtually all cultures as a source of medicine. Interest in herbal drugs is rowing due to their efficiency, low toxicity and absence / minimal side effects (Saumya et al., 2011).

*Aristolochia indica* is a wein in nature commonly called as “Perumarundhu” in Tamil. In Sivagangai district the plant has been reported to possess several medicinal uses which include anti - dote and this plant syrup combine with honey used to treat children who suffer dry cough and fever. The aim of this study was to investigate the Nephroprotective activity of *Aristolochia indica* leaves on gentamicin induced nephrotoxicity in rats.

**MATERIALS AND METHODS**

**Animals:**

Male albino rats of Wistar strain approximately weighing 180-200g 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 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water. They were adopted to the environment for one week prior to experimental use.

**Chemicals:**

Gentamicin, Sodium hydroxide and Trichloro Acetic acid (TCA) and were purchased for Sigma chemical company, Mumbai. All other chemicals and reagents used in this study with high purity were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai.

**Plant Material and Preparation of Extract:**

The leaves of *Aristolochia indica* were collected from Mayiladuthurai & Thanjavur on January 2014. The collected leaves of *Aristolochia indica* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *Aristolochia indica* was macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45ºC. A semi solid extract was obtained after complete elimination of alcohol. The residue was kept in the refrigerator for further use. The extract was dissolved and made up to a known volume using distilled water just before the extract orally given to the rats.

**Experimental Design:**

Body weights of animals were recorded and they were divided into three groups. Each group contains six animals.

- **Group I:** Control animals received with standard fed and water.
- **Group II:** Received intraperitoneal injection of gentamicin (40mg/kg Bodyweight) daily for two consecutive weeks.
- **Group III:** Treatment group received gentamicin(40mg/kgb.wt.) and co-treatment with ethanolic extract of *Aristolochia indica* leaves at a dose of 500mg/kgo.p (Aqueous) body weight for two weeks.

**Collection of Blood and Preparation of Serum Sample:**

At the end of the experimental period, the animals were anaesthetized using chloroform vapour prior to dissection. Blood was collected without EDTA in the test tubes. The blood 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 10minutes, and then the serum (supernatant) was isolated and stored refrigerated until required.

**Tissue Homogenate:**

Immediately after blood collecting, the animals were sacrificed by cervical dislocation and the kidney was dissected out, washed with ice-cold physiological saline. The required tissues were weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris HCl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

**Biochemical Analysis:**

Malondialdehyde (MDA) was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Reduced glutathione was estimated by method of Moron et al.,(1979). Serum sodium was estimated by colorimetric method of Maruna & Trinders (1958). Serum potassium was estimated by method of Maruna (1957). Urea was estimated by the method of Natelson (1957). Serum creatinine was carried out by alkaline picrate method C Bonses and Taussy (1954). Protein was estimated by the method of Lowry et al., in 1951. The activity of mitochondrial glutathione peroxidase was assayed by the method of Rottruck et al., (1973). Superoxide dismutase activity was determined by the procedure of Kakkar et al., (1984). The activity of catalase was assayed by the method of Beers and Sizer (1952).

**Statistical analysis:**

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences...
between mean values were determined by one way analysis of variance (ANOVA) followed by the multiple comparisons. The results were statistically analyzed by Graphpad Instant Software (Graphpad Software, San Diego, CA, USA) version 3 and p< 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Kidneys endowed with million units are termed as nephrons that act as a 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 (Lesely and Levey, 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, (Asiiley, 2004). 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 Aristolochia indica on gentamicin-induced rats.

Gentamicin (GM) is widely used aminoglycoside 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 (Paller et al., 1984). GM was reported to enhance the generation of hydrogen peroxide and oxygen free radicals (Yukawa O & Nagzaka 1980). Reactive oxygen species may produce cellular injury and necrosis via., several mechanisms including peroxidation of membrane lipids, protein, denaturation and DNA damage (Gutteridge, 1992). In this context a marked increase in the concentration of serum MDA were observed in gentamicin induced rats when compared to control rats (Fig. 1). Administration of Aristolochia indica significantly decreased the levels of MDA in gentamicin-induced rats. This view is supported by Barrouillet et al. (1999) studies.

Glutathione is a ubiquitous thiol containing tripeptide, which plays a central role in cell biology. It is implicated in the cellular defence 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 (Pastore et al., 2003). 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 (Fig. 1). Supplementation of Aristolochia indica 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 prevent 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). Enzymatic antioxidants such as SOD, GPx and Catalase are free radical scavengers. Their synergistic action in scavenging oxygen-derived free radicals is well documented (Wojacki et al., 1995).

Biological systems protect themselves against the damaging effects of activated species by several means. These include free radical scavengers and chain reaction terminators; enzymes such as SOD, CAT and GPx system. The SOD dismutates superoxide radicals O2- into H2O2 plus O2 which participating with other antioxidant enzymes, in the enzymatic defense against oxygen toxicity. CAT is a key component of the antioxidant defense system and catalyse the reaction of hydrogen peroxide into water... Inhibition of these protective mechanisms results in enhanced sensitivity to free radical-induced cellular damage. Excessive production of free radicals may result in alterations in the biological activity of cellular macromolecules. GPx is a is a seleno-enzyme two third of which is present in the cytosol and one-third in the mitochondria. It catalyses the reaction hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Halliwell B, Gutteridge, 1999).The activities of Kidney SOD, GPx and Catalase in the present
Fig 2 Effect of Aristolochia indica on Protein, Urea and Creatinine in experimental rats

Fig 3 Effect of Aristolochia indica on sodium in experimental rats

Fig 4 Effect of Aristolochia indica on SOD, Catalse and GPx in experimental rats

study were significantly reduced in gentamicin treated rats than in the experimental control rats (Fig. 4). Decrement in the activity of renal SOD, GPx and CAT following GM treatment are in accordance with previous report on GM induced suppression of endogenous enzymatic antioxidant machinery (Thounaojam et al., 2010). Supplementation with Aristolochia indica to gentamicin treated rats resulted in near normal activity of SOD, GPx and CAT. Our results are companionable with the study of Ashraf et al. (1999).

Urea is a 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 (Lesley and Levey, 2005). Drugs that can increase urea levels include allopurinol, some diuretics, gentamicin and indometacin (Mason, 2004). In the present study also observed the increased level of urea in gentamicin intoxicated rats. Supplementation of Aristolochia indica restored the increased level of urea in gentamicin-induced rats (Fig. 2). This result is support by Kotnis et al. (2004) studies.

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) (Perrone et al., 1992). The serum creatinine concentration is the most commonly used index of the kidney function. The level of creatinine is the blood rises if the kidney does not function properly (Lesley and Levey, 2005). Gentamicins have been reported to increase creatinine measurements (Mason, 2004). This result supports our findings (Fig. 2). Administration of Aristolochia indica restored the level of creatinine in gentamicine treated rats. This study is consisted with and Kotnis et al. (2004) studies.

Proteinuria, most often reflecting loss of the normal glomerular impermeability to filtration of plasma proteins, is an early sign of kidney disease. Impairment of the normal capacities of various segments of the renal tubules to reabsorb water and electrolytes to effectively maintain the volume and composition of body fluids within normal limits ma also be key manifestations of kidney dysfunction. Proteinuria is a hallmark of kidney disease. Thus detection of proteinuria is necessary for the recognition of most kidney diseases (Cohen and Lemann, 1991). In the present study, protein was found to be decreased significantly gentamicin induced rats when compared to normal rats. The decreased level of protein observed in gentamicin treated rats (Fig. 2) may be due to toxicity of free radical generated from gentamicin which damaged nephron and thereby loss of protein through urine. Administration of Aristolochia indica normalized the level of serum kidney protein in gentamicin treated rats. This result is lending with Kotnis et al. (2004) studies.

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 (Cohen and Lemann, 1991). In the present study, gentamicin treated rats significantly lower in potassium levels (Fig. 1) and higher in sodium levels (Fig. 3) 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 Aristolochia indica restored the normal level of sodium and potassium in gentamicin treated rats. This result consisted with Silan et al. (2007) studies.

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

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