

## EFFECT OF *IPOMEA OBSCURA* LINN IN NEPHROTOXIC INDUCED IN EXPERIMENTAL RATS

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### ABSTRACT

The present study has been undertaken to evaluate with the nephroprotective effect of crude powder of *Ipomea obscura* Linn leaves in normal and gentamicin induced nephrotoxic rats. Gentamicin was used to induce nephrotoxicity in male albino rats weighing between 100-120gms for 30 days. Effects of the plant crude powder treatment on the hematological and renal histological profile in gentamicin nephrotoxic rats were also evaluated. *Ipomea obscura* used in Siddha system of medicine and the plant is known to contain various active principles of therapeutic value and to possess biological activity against a number of diseases.

**KEYWORDS:** Renal failure, Albino rats, *Ipomea obscura* L, Urea, Uric acid, Creatinine, phosphatases and transaminases and Cystatin C.

### INTRODUCTION

The World Health Organization has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of years (Kamboj VP,2000). The traditional preparations comprise medicinal plants, minerals and organic matter. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy (Hota and Pathi, 2003). Siddha medicine is essentially promotive and preventive in therapeutic approach.

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 (Arias *et al.*, 2003). The Chronic symptoms include: Poor appetite, Vomiting, Bone pains, Headache, Stunted growth, Malaise, High urine output or no urine output, Recurrent urinary tract infections, Urinary incontinence, Pale skin, Bad breath, Hearing deficit, Detectable abdominal mass, Tissue swelling, Irritability, Poor muscle tone, Change in mental alertness (Perneger *et al.*, 1994).

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 *Tribulus terrestris*, *Marsilea quadrifolia*, *Hybanthus enneaspermus*, *Clitorea ternatea*, *Hygrophila auriculata* etc.

Drug-induced nephrotoxicity is an important cause of renal failure. Aminoglycosides throughout the endocytic pathway are taken up into the epithelial cells of the renal proximal tubules and stay there for a long time, which leads to nephrotoxicity. Acidic phospholipids, broadly distributed in the plasma membranes in various tissues, were considered to be the binding site of aminoglycosides in brush-border membrane of proximal tubular cells (Nagai and Takano, 2004 and Nagai, 2006). Hydroxyl radicals play a role in the pathogenesis of gentamicin nephrotoxicity, gentamicin can induce suppression of Na(+)-K(+)-ATPase activity and DNA synthesis in rats proximal tubules leading to renal injury; this injury may be relevant to reactive oxygen metabolites generated by gentamicin. Renal cortical mitochondria are the source of reactive oxygen metabolites, which induces renal injury (Nephrol Dial Transplant, 1994).

Proposed pathological mechanisms of gentamicin induced nephrotoxicity include induction of oxidative stress, apoptosis, necrosis, elevation of endothelin I, and increase of monocyte/ macrophages infiltration. Gentamicin-induced nephrotoxicity is characterized functionally by increased serum creatinine, increased blood urea nitrogen, and decreased glomerular filtration rate and morphologically characterized by proximal tubule epithelial desquamation, tubular necrosis, epithelial edema, and glomerular hypertrophy (Bledsoe *et al.*, 2006).

Biochemical markers play an important role in accurate diagnosis and also for assessing risk and adopting therapy that improves clinical outcome. Over decades research and utilization of biomarkers has evolved substantially. National Institute of Health (NIH) 2001 defined a biomarker as “a characteristic that is objectively measured and evaluated as an indicator of normal biological, pathologic processes, or pharmacologic responses to a therapeutic intervention. For renal disease urea, creatinine, uric acid and cystatin C are key renal markers for assessing the pathological condition. Measurements of biomarkers in the blood or urine that detect patients at risk of kidney diseases, or that detect kidney diseases in the earliest stage, may ultimately result in preventive, earlier or more effective treatments for kidney diseases (Naicker,2011).Cystatin C is a small molecule (13kDa) that is filtered and metabolized after tubular absorption (Randers and Erlandsen,1999 and Madero *et al.*, 2006).It is a sensitive biomarker of kidney function in mild-to-moderate kidney disease (Newman *et al.*,1995).

### ***Ipomea obscura* L**

*Ipomoea obscura* (L.) Ker-Gawl (Family: Convolvulaceae) is a slender, twinning perennial herb found almost throughout India upto an altitude of 3000 ft., in grasslands, hedges and waste lands (Kirtikar and Basu, 1999). In Uganda, it is used for the treatment of diarrhea by traditional healers (Anokbonggo, Odoi-Adome and Oluju, 1990). Leaves of this plant are used as an application to aphthous affections after toasting, powdering and boiling with ghee and in admixture with the leaves of *Argyreia mollis* for sores (Kirtikar and Basu, 1999). Chemical investigations of this plant have shown the presence of tropane alkaloids such as Calysteginine B-1, Calysteginine B-2, Calysteginine B-3, Calysteginine B-4 and Calysteginine C-1 and indole alkaloids such as Ipobscurine A, Ipobscurine B, Ipobscurine C and Ipobscurine D (Asano and Yokoyama, 2001). Plants belonging to *Ipomoea* species such as *Ipomoea batatas* (L.) have shown significant antioxidant activity (Dong *et al.*, 2004). No biological activity, however, is reported for *Ipomoea obscura* (L.) till date. Hence, in the present investigation different extracts of the whole plant, *Ipomoea obscura* (L.), were screened for their in vitro antioxidant activity using standard procedures.

## EXPERIMENTAL METHODS

### Plant Material

Fresh plant sample *Ipomea obscura* L were collected from various parts of Thanjavur district. The whole plant were washed, shade dried, powdered. Crude powder of *Ipomea obscura* L are given to the experimental rats at the dose of 100mg/100g body weight.

### Experimental Design

Male albino rats of 8 – 10 weeks of age weighing between 100 and 120g were used for the study. The animals were housed in polypropylene cages. Animals were divided into three groups of 5 animals. The animals were acclimatized for a week under laboratory conditions. All experiments were performed according to the norms of the local ethical committee.

Renal damage was induced in rats by administering Gentamycin at a dose of 40mg/100kg of body weight intraperatonially.

Experimental animals were distributed randomly, in three groups, containing three animals each.

*Group-I : The animals in group I served as control and received rat feed and distilled water.*

*Group-II : The group II rats served as test and were administered with gentamycin at a dose of 40 mg/ 100g body weight intraperatonially.*

*Group-III : The animals provided with rat feed and distilled water along with gentamicin and Crude powder of Ipomea obscura at the dose of 100mg/100g body weight the drug followed by it.*

After the completion of the experimental regimen rats were fasted overnight, anaesthetized with ether, blood was drawn and the serum was separated for various biochemical parameters. Kidney samples are also collected for the studies.

### Biochemical Studies

Nephroprotective activity was done by assessing the significant changes in body weight, blood urea, serum creatinine, uric acid, cystatin C, phosphatases and transaminases. Serum creatinine level was determined using Creatinine Colorimetric Kit. Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interference. (Bartels and Bohmer, 1971; Fabiny, Ertingshausen, 1971). In the estimation of Urea, diacetyl monoxime

in the presence of acid hydrolyzes to produce the unstable compound diacetyl reacts with urea to produce a yellow diazine derivative. The colour of this product is intensified by the addition of thiosemicarbazide it was measured at 520nm (Cocilombe and Crocker, 1967)

Uric acid reduces phosphotungstic acid in the presence of sodium carbonate to give blue colour which can be measured colorimetrically. Transaminases activities were estimated by Reitman and Frankel method and which was measured spectrometrically. The acid phosphatases was estimated and the absorbance was read at 405nm (Andersch, 1974). Cystatin C is estimated in serum using the kit and measured at 700/546 nm. The Histopathological studies, were carried out in all the groups of normal, control and drug treated rats (Ochei and Kolhatkar., 2000).

Mean values standard were calculated for all the values carried out. Based on this student 't' test were analyzed and probability of error was assessed (Fisher, 1950).

## RESULTS AND DISCUSSION

Gentamicin (GM) is an aminoglycoside antibiotic that is very effective in treating life threatening gram negative infection. GM induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubule. The specificity of the gentamicin for renal toxicity is apparently related to its preferential accumulation in the renal proximal convoluted tubules (50 to 100 times greater than serum).

GM causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure (Sudin, 2001).

The result of this study shows the significant nephrotoxicity induced by gentamicin was evidenced by increase in serum urea, creatinine clearance and urea secretion due to renal tubular necrosis. The administration of crude powder of *Ipomea obscura* for 30 days was found able to treat and protect renal necrosis against gentamicin induced nephrotoxicity and thereby decreasing the serum urea, creatinine and uric acid.

In the present experimental serum cystatin C level was decreased from the untreated control animals. In drug induced nephrotoxicity there was an increase in serum cystatin C due to the impaired glomerular filtration. Cystatin C appears to be a better predictor of glomerular function than serum creatinine. Cystatin C, a non-glycosylated 13 kDa protein,

has the potential to improve estimates of GFR, because it is thought to be less influenced by muscle mass or diet. Glomerular Filtration rate is estimated with Cystatin C. (Table:1)

Aminotransferases (ALT and AST) and Phosphatases (Table:2) are the specific enzymes and are considered to be very sensitive and reliable induces for measuring hepatotoxic as well as protective effect of various compounds. Renal necrosis induced by gentamicin usually associated with elevated levels of serum enzymes that are indicative of cellular leakage and loss of functional integrity of cell membrane in kidney. (Reitman and Frankel, 1957).

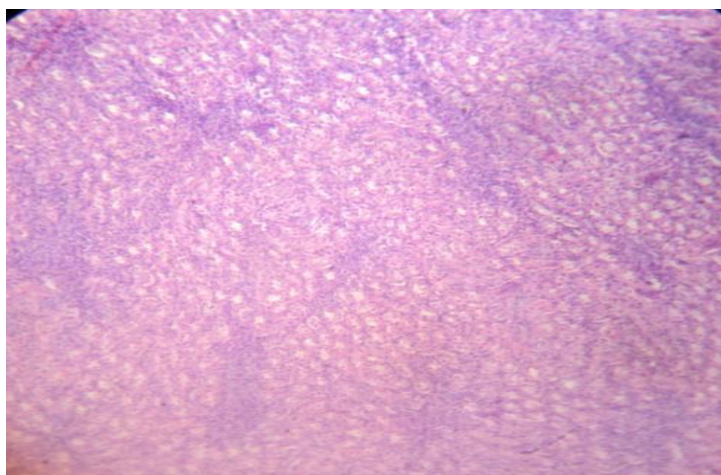
The oral administration of crude powder of siddha medicinal plant *Ipomea obscura* for 30 days were found to protect the proximal tubular damage induced by lipid per oxidation and activation of antioxidant enzymes. The drug administration was able to protect the renal necrosis and lysosomal latency as evidenced by the inhibitory activity of phosphatases and transaminases. The study also shows the significant efficacy of herbs in the treatment of nephrotoxicity was also evidenced by decrease in urea level and creatinine clearance.

The nephroprotective effect of be due to the activity of the phytoconstituents present in the Siddha medicinal plant *Ipomea obscura* have the nephrotoxic effect against the aminoglycoside-antibiotic drug gentamicin. Further studies are suggested on the isolation of active compounds and their nephroprotective activity in human.

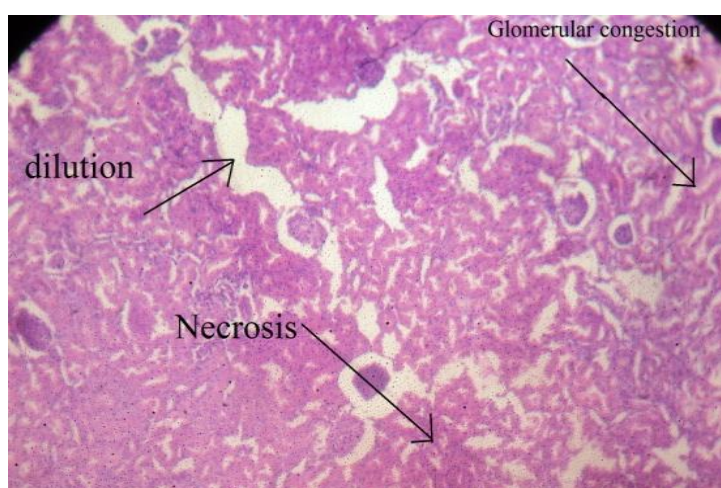
### **Histopathology**

In histopathological examination, normal architecture was observed in control animals whereas renal lesions including marked tubular and focal area necrosis, inflammation and glomerular congestion changes in the kidney of gentamicin treated animals were observed (Group 2- control). The lesions were reduced significantly in animals which were treated with the Siddha medicinal plant *Ipomea obscura* at the dose of 100 mg/100g.b.wt (dosess) to gentamicin treatment.

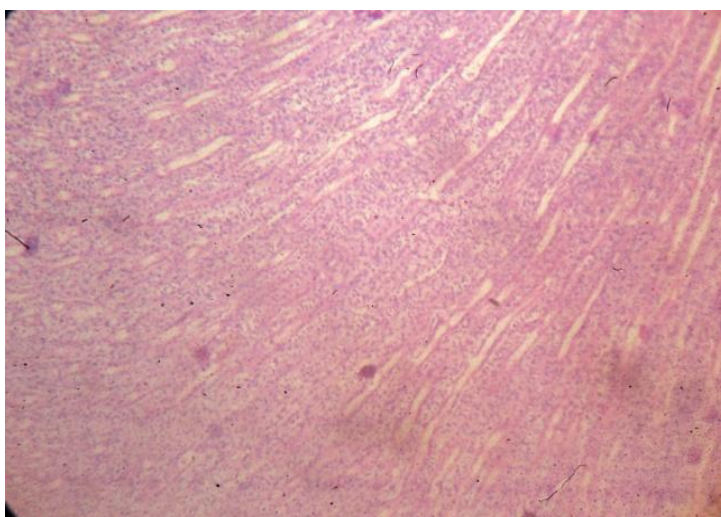




**Group 1 –Normal**



**Group 2 – Control**



**Group 3 – Drug treated**

**TABLE 1: Nephroprotective effect of *Ipomea obscura* on biochemical parameters- Urea, Uric acid, Creatinine and Cystatin C.**

Groups	Dose	Parameters			
		Urea mg/dl	Uric acid mg/dl	Creatinine mg/dl	Cystatin C mg/l
Normal	Isosaline	2.81±2.24	3.8±3.04	1.4±0.56	1.59±0.79
Control	40mg/kg b.wt	5.66±3.39	6.7±4.69	2.62±1.57	4.82±3.85
Drug Treated	100mg/kg b.wt	3.29±2.63	4.24±2.12	1.56±0.78	3.66±0.73

**TABLE 2: Nephroprotective effect of *Ipomea obscura* on Phosphatases and Transaminases.**

Groups	Dose	Parameters		
		Acid Phosphatase U/l	Alanine Transaminase U/l	Aspartate Transaminase
Normal	Isosaline	15.71±9.42	13.76±9.63	10.75±7.52
Control	40mg/kg b.wt	19.23±3.84	17.21±3.44	18.11±9.05
Drug Treated	100mg/kg b.wt	13.14±2.62	12.17±2.43	13.17±3.95

Each value is the mean ± SEM of five samples values are significantly different from control and treated rats.

## CONCLUSION

The crude powder of the plant *Ipomea obscura* L has nephroprotective activity against the gentamicin induced nephrotoxic rats. Further studies, are needed to identify the chemical constituents of the plant *Ipomea obscura* L that may be responsible for the nephroprotective activity.

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