Research Article

Nephrotoxicity by Using Gentamicin in Rats and Screening of *Simarouba gluaca* Extract for its Nephroprotective Activity

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ABSTRACT

The aim of the current study was to evaluate in-vivo Nephroprotective activity of *Simarouba glauca* against gentamicin induced Nephrotoxicity in albino Rats. The *Simarouba glauca* were dried under shade and then powdered, and extracted with 90% methanol by reflux. Preliminary phytochemical studies and acute toxicity studies were also carried out. Nephrotoxicity was induced in rats by i.p. injection of gentamicin at a dose of 80 mg/kg body weight for 10 days. Group A served as normal control while group B was considered as disease control. Group C was standard receiving Vitamin E 250 mg/kg and Group D disease animals were treated with *Simarouba glauca* (250 mg/kg and 500 mg/kg). The parameters included in this study were serum uric acid Creatinine, BUN, total protein, phosphorous and glucose also were analyzed as part of this study. Apart from this, Histopathological studies of kidney were also conducted. The results showed that when treated with *Simarouba glauca*, elevated serum uric acid, serum Creatinine levels with gentamicin administration were bought back to normal values. Results of histopathological studies also showed that *Simarouba glauca* administration noticeably reduced the gentamicin induced renal damage. Significant Nephroprotective activity of *Simarouba glauca* observed in the present investigation could be the result of decreased the biochemical parameters. Methanolic extract of Simarouba glauca also showed improvisation in kidney function and may have protective effect in nephrotoxicty related complications.

Keywords: *Simarouba glauca*, Gentamicin, Nephroprotective.

INTRODUCTION

Across history and cultures around the world, plants have been used as medicines. Around the world, a large population still access herbal remedies to treat the illness. Since a large portion of the world population is getting aged, now there is a greater attention regarding the chronic illness which causes the health care budgets to rose. Botanical medicines might help to ameliorate the chronic illness and assists in optimizing the health of aging population by natural means.[1]

Research on medicinal plants is one of the key areas which attract the researchers globally as there is an increase in demand for herbal remedies in both developed and developing countries. However, safety evaluation, bioactivity and conservation of medicinal plants are some of the key areas that need close attention.[2]

Nephrotoxicity is a renal dysfunction or failure occurs as a result of exposure to external agents such as chemicals and drugs. Many therapeutic agents have been identified as materials that induce nephrotoxicity.[3] Disruption of normal cellular functions of mitochondria or intra tubular obstruction like crystal disposition which causes renal injury, results in developing nephrotoxic effect in glomerular and tubular epithelial cells. Effects of medicines also cause chronic renal failures and it leads to chronic interstitial injury and papillary necrosis.[4] High renal excretion as an effect of some medicines cannot be treated as nephrotoxicity, rather proper dose adjustment needs to take care for proper renal function.

Kidney is an important excretory organ in the human body. Important functions of kidney includes excretion of the metabolic waste products through urine, maintain the acid base balance and endocrine functions like erythropoietin production.[5]

So nephrotoxicity is becoming a major concern in drug development and discovery. Worldwide, number of persons infected with kidney disorder is increasing. Kidney disorder statistics for the United States conveys the alarming picture of chronic kidney disease (CKD) and end-stage renal disease (ESRD), researchers already started to identify the need for resources such as transplant clinics and dialysis equipment's to treat the growing number ESRD population.[6,7]

Worldwide, it is estimated that number of people surviving on dialysis already crossed a million. Among the diseases that leads to kidney failure, diabetes is considers being the most important. Diabetes is very common in Asia and the number of diabetes patients in Asia is about five times than the white population. Hypertension, a lifestyle disorder is another major reason for kidney failure. In the last few decades there is an increasing trend of people reporting kidney failure due to hypertension. Again, Asian population is vulnerable in this case too, which is twice when compared to the white population. Among the kidney failures reported, 66% is either by diabetes or hypertension. In India around 7.85 million patients are suffering from CRF, in which, 41% is

caused due to diabetes while 22% are the result of hypertension. In United States number of people treating for CRF is around 30 million .[8]

Traditionally all over the world herbs are used for treating drug induced kidney disorders. Normally herbal plats are easily accessible to people and they are cheap also herbal plants possess minimal side effects. In the recent years, researchers around the world are interested in the evaluation of the effects of traditional medicines and herbs. Nephrotoxicity and its protection is also one of the key areas where much of such research is happening. Already there are many promising outcome which reaffirms the effectiveness of several herbs and herbal extracts in clinical evaluation. Even though several plants are evaluated against kidney failures, fair research was happened only against a few of them.

Hence, the aim of the present study was to examine the Nephroprotective effect of *simarouba gluaca* on important nephrotoxic drug such as gentamicin. In this study (90%v/v) hydro alcoholic extract of *simarouba gluaca* was evaluated thoroughly for Nephroprotective activity against Gentamicin induced nephrotoxicity in albino rats.

MATERIAL AND METHODS

Collection and authentication of plant material:

The fresh leaves of *Simarouba glauca* were collected and authenticated by Botanist, Bangalore.

Preparation of 90% v/v methanolic extract of *Simarouba glauca* [9]

The leaves of *Simarouba glauca* was chopped into small pieces and dried under shade at room temperature for seven days. The dried leaves were powdered and passed through the sieve (coarse10/40). The powder was used for the preparation of methanolic extract. Dried and powdered leaves of *Simarouba glauca* (each 1.0 kg) were extracted with boiling 90% MeOH in a reflux condition. After filtration, the solution was concentrated under a vacuum.

Phytochemical analysis of *Simarouba glauca*

Preliminary qualitative analysis of *Simarouba glauca* was analysed qualitatively.

Experimental animals

Wistar rats weighing 150-200g were used for the experiment. They were acclimatized for one week prior to experiment. Animals were caged in fully ventilated room, were maintained in 12:12 h light and dark cycle and were housed at temperature of 25 ± 2°C. They had free access to a standard chow diet and water *ad libitum*. All the experiments conducted on the animals were in accordance with the standards set for the use of the laboratory animal use and the experimental protocols were duly approved by the IAEC (Institutional Animal Ethical Committee) of Karnataka College of pharmacy, Bangalore.

Experimental Design

Acute Oral Toxicity Study

The acute oral toxicity study was performed according to the OECD guidelines no. 425.

Nephroprotective activities

Model I

Evaluation of nephroprotective activity of *Simarouba glauca* in gentamicin induced nephrotoxicity

Age matched young albino rats weighing about 150-200 g were employed in the present study. Rats were fed on standard chow diet and water ad libitum. The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. They were acclimatized in institutional animal house and were exposed to normal cycles of day and night. Gentamicin (80 mg/kg/day) intraperitonially was administered for 10 days to induce experimental nephrotoxicity in rats. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in polyethylene cages. The rats were divided into following groups consisting of six rats each.

Goup1:	Administered vehicle serves as Normal control.
Goup2:	Administered Gentamicin (80mg/kg i.p.) Serves as disease control for 10 days.
Group3:	Administered Reference standard, VitE (250 mg/kg) for 10 days.
Group4:	Diseased rats treated with methonolic extract of *Simarouba glauca* (250mg/kg, p.o. once daily for 10 days).
Group5:	Diseased rats treated with Methonolic extract of *Simarouba glauca* (500mg/kg, p.o. once daily for 10 days).

At the end of experimental period all the animals were anesthetized using high dose of Phenobarbital for tissue histology. Blood collected by Cardiac puncture was centrifuged at 2500 rpm for 15 minutes and analyzed for various biochemical parameters such as glucose, serum urea, Creatinine, calcium, BUN, uric acid, phosphorous and total protein.

Histopathological Studies

Preparation of isolated kidney

The animals were euthanized using high dose of pentobarbital and sacrificed. And the kidney of each animal was isolated and was cut into small pieces, preserved and fixed with 10% formaldehyde. The samples were then dehydrated and embedded in paraffin. After

sectioning (5µm thick) with a rotary slicer (LEICA RM2135, Wetzlar, Germany), hematoxylin and eosin stain (H&E).

Statistical analysis

The results are expressed as mean ± S.D from n=6 rats in each group. The significance of difference among the groups was assessed using one-way analysis of variance (ANOVA) followed by Tukey's test compared between Normal control (Untreated) vs all groups $p<0.05$ were considered significant.

RESULTS

Simarouba glauca was analyzed qualitatively. It was observed that the extract may contain Alkaloids, Phenols, Tannins, Flavonoids, cardiac glycosides, carbohydrates, saponin and triterpenoids.

Acute toxicity study

The LD_{50} of the extract of *Simarouba glauca* was found to be 5000 mg/kg after conducting the acute toxicity studies. So $1/10^{th}$ and $1/20^{th}$ of dose was selected and the experiment was carried out.

Table 1: Preliminary qualitative analysis of leaves of *Simarouba glauca*

S.N.	Phytochemical constituents	Observation
1	Alkaloids	+
2	Phenols	+
3	Tannins	+
4	Flavonoids	+
5	Anthraquinones	-
6	Cardiac glycosides	+
7	Carbohydrates	+
8	Saponins	+
9	Triterpenoids	+

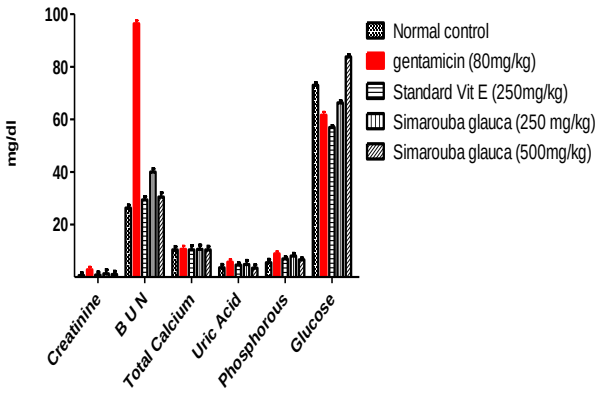
Present (+) or absent (-)

Table 2: Effect of oral administration of methanolic extract of *Simarouba glauca* (250mg and 500mg/kg.po/day/10days) on gentamicin (80mg/kg.ip/10 days) treated rats on serum biochemistry level after 10 days of treatment

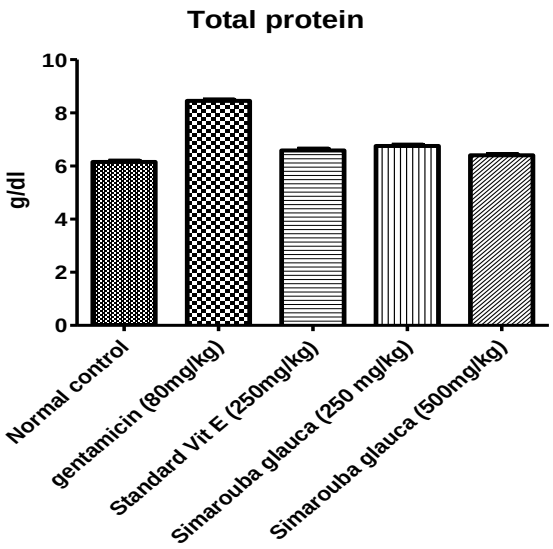
Test/Parameters	Normal control	Gentamicin (80mg/kg)	Standard Vit E (250mg/kg)	*Simarouba glauca* (250 mg/kg)	*Simarouba glauca* (500mg/kg)
Creatinine (mg/dl)	0.60±1.031	2.80±1.100***	0.80±1.070$^{###}$	1.30±1.520†††	1.00±1.190†††
B U N(mg/dl)	26.30±1.050	96.40±1.210***	29.40±1.234$^{###}$	40.00±1.230†††	30.50±1.660†††
Total Calcium(mg/dl)	10.39±1.201	10.60±1.277***	10.40±1.540$^{###}$	10.60±1.570†††	10.40±1.244†††
Uric Acid(mg/dl)	3.57±1.230	5.70±0.990***	4.58±0.850$^{###}$	4.80±1.511†††	3.40±1.220†††
Phosphorous(mg/dl)	5.50±1.250	8.87±0.890***	6.90±0.780$^{###}$	8.03±0.960†††	6.60±0.680†††
Glucose(mg/dl)	73.00±0.903	61.60±1.240***	56.90±0.680$^{###}$	66.30±0.820†††	83.80±0.776†††
TOTAL PROTEIN (gm/dl)					
	6.15±0.0428	8.45±0.0428***	6.58±0.0600$^{###}$	6.75±0.0428†††	6.40±0.0365†††

Values are expressed as mean ± SEM, n = 6 in each group. $^{***}P < 0.001$.when compared to control group. $^{###}P < 0.001$ when compared to preventive diabetic control group. $^{†††}P < 0.001$ when compared to gentamicin control group and with their respective group

Gentamicin treated group displayed significant increase ($P<0.001$) in serum Creatinine, uric acid, and electrolytes when compared with the normal control group. While animals treated with *Simarouba glauca* (250 and 500mg/kg b.w., p.o.) along with the administration of Gentamicin showed dose dependant significant decrease ($P<0.001$) in serum Creatinine, uric acid, and electrolytes when compared with Gentamicin treated group. These values are tabulated in the Table 02 and graphically represented in Fig 01.



Values are expressed as mean ± SEM, n = 6



Values are expressed as mean ± SEM, n = 6

Histological examination of haematoxylin and eosin (H&E) stained on Kidney tissue

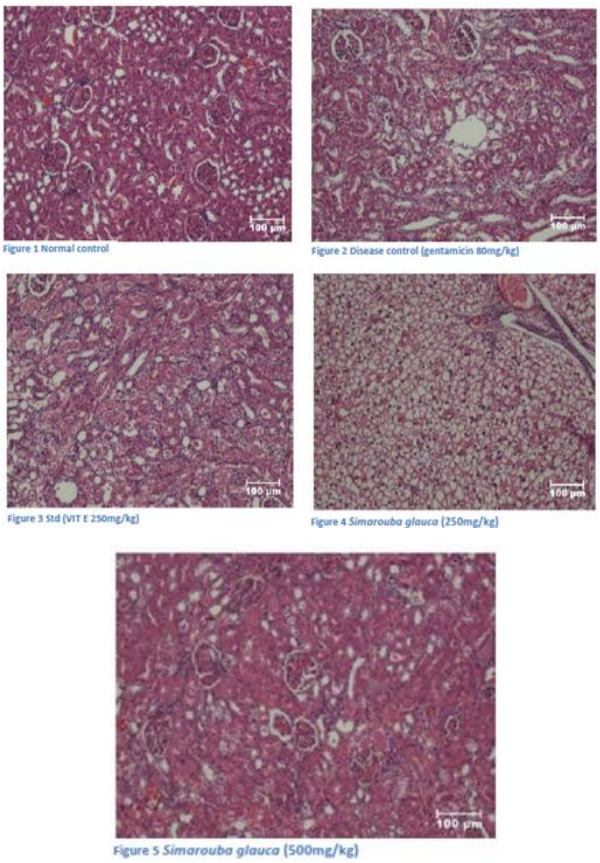


Figure 2: Effect of oral administration of methanolic extract of *Simarouba glauca* (250mg and 500mg/kg.po/day/10days) on gentamicin (80mg/kg.ip/10 days) treated rats on tissue after 10 days of treatment

Normal control-Kidney showing normal glomerulus with tuft of capillaries surrounded by Bowman's capsule with tubules lined by columnar epithelial cell cytoplasm staining pink colour and basal nucleus blue in colour with normal architecture. Haematoxylin and Eosin stain, scale bar = 100µm. Disease control gentamicin Kidney showing glomerular degeneration with loss of capillaries surrounded by Bowman's capsule. The tubules showed nephrotoxicity with severe tubular degeneration and loss of tubular architecture which also evident by accumulation in the centre of the tubules. Haematoxylin and Eosin stain, scale bar = 100µm. Standard drug vit E Kidney showed glomerulus with loss of capillaries surrounded by Bowman's capsule. The tubules showing recovery from nephrotoxicity and appears to be normal architecture with mild tubular degeneration evident by accumulation in the centre of the tubules. Haematoxylin and Eosin stain, scale bar = 100µm. Low dose of *Simarouba glauca* Kidney showed recovery and normal architecture of glomerulus with tuft of capillaries surrounded by Bowman's capsule. The most of tubules are showing normal architecture and recovery. However, few tubules showed mild degeneration evident by accumulation in the centre of the tubules. Haematoxylin and Eosin stain, scale bar = 100µm. High dose of *Simarouba glauca* Kidney showed recovery and normal architecture of glomerulus with tuft of capillaries surrounded by Bowman's capsule. The tubules showed normal architecture and recovery. Haematoxylin and Eosin stain, scale bar = 100µm.

DISCUSSION

Nephrotoxicity is a poisonous effect of substances such as medicines and toxic chemicals on the kidneys. Drugs such as anti-cancer drugs, amino glycosides, NSAIDs, antibiotics and ACE inhibitors are potential Nephrotoxins. Nephrotoxic effect may advances in glomerular and tubular epithelial cells as result of mechanism that rattles the membrane integrity or normal cellular functions of mitochondria induced renal injury.

Even though there is no particular treatment of drug induced nephrotoxicity, previous studies reports revealed that there are some prophylactic treatments to reduce the nephrotoxicity.[50] Present study was an attempt to screen nephroprotective activity of methanolic (90% v/v) extract of *Simarouba glauca* has been reported with many medicinal uses. The present study was aimed to evaluate the nephroprotective effect of *Simarouba glauca* against gentamicin induced nephrotoxicity in rats. Preliminary qualitative phytochemical studies of *Simarouba glauca* revealed the presence of Alkaloids, Phenols, Tannins, Flavonoids, cardiac glycosides, carbohydrates, saponin and triterpenoids.

Acute toxicity study was conducted according to OECD guidelines. Single dose administration of *Simarouba glauca* at 5000mg/kg b.w P.o showed no mortality in any of the animals used. Hence 250mg/kg b.w and 500mg/kg b.w of maximum dose were selected for the present study. Gentamicin 80mg/kg at a dose of 80mg/kg b.w, i.p. for 10 days in rats was adequate to cause nephrotoxicity. Lysosomal phospholipase-A2 plays a key role mechanism of gentamicin 80mg/kg induced nephrotoxicity. At the

renal brush border, inhibition of lysosomal phospholipase-A2 causes the lysosomal rupture and release of acid hydrolases; this leads to entry of amino glycosides in to cytosol. This free drug displaces the Ca^{2+} when binds with mitochondria and causes mitochondrial degeneration and necrosis. Oxidative stress (OS) is another important factor with key role in the nephrotoxicity of gentamicin. In the presence of metal catalysts, hydrogen peroxide and superoxide anions react to form hydroxyl radical. As this hydroxyl radical mediates the OS, mitochondrial DNA (mtDNA) is much vulnerable to oxidative damage with the increased presence of free radicals and super oxides such as ROS in the mitochondrial matrix[10-12].

Gentamicin 80mg/kg is associated with wide range of metabolic disorder including uric acid and Creatinine disparity so that finding the amount of these parameters are considered to be a reliable methods for diagnosing drug induced nephrotoxicity. In the present study demonstrated that gentamicin 80mg/kg induced renal injury as evident from elevated serum uric acid and Creatinine. This might be due to destruction of tubular epithelial cells or decreased GFR rate or dehydration. When compared with control animals; gentamicin 80mg/kg treated animal showed changes in physical and biochemical parameters such as significant decrease in serum biochemical parameters. Animals treated with *Simarouba glauca* provide protection against gentamicin 80mg/kg induced nephrotoxicity by all the physical and biochemical parameters brought back to near normal depending upon the dose. Gentamicin treated group displayed significant increase ($P<0.001$) in serum Creatinine, uric acid, and electrolytes when compared with the normal control group. *Simarouba glauca* (250 and 500mg/kg b.w., p.o.) along with the administration of Gentamicin showed dose dependant significant decrease ($P<0.001$) in serum Creatinine, uric acid, and electrolytes when compared with Gentamicin treated group. Histological evidence showed a gentamicin 80mg/kg treated rats developed tubular necrosis, morphological changes in mesangial cells of glomerulus and interstitium showed scattered mononuclear inflammatory infiltration with decrease in stromal cells. Treatment with *Simarouba glauca* diminishes the gentamicin 80mg/kg induced damages in dose dependent manner. The study revealed that the methanolic extract of *Simarouba glauca* protect the kidney from toxicity induced by chemical compounds.

CONCLUSION

The present study indicated that administration of Methanolic extract of *Simarouba glauca* at the dose of 250 and 500mg/kg b.w. Possess Nephroprotective activity in gentamicin induced nephrotoxicity in rats. The acute toxicity study indicated that the extract was devoid of major toxic effect. The Nephroprotective effect of *Simarouba glauca* was confirmed by preventing the reduction in serum biochemistry on gentamicin treated rats. Beside this Histopathological studies concluded that animal treated with *Simarouba glauca* decreased the gentamicin induced renal damage. Hence all the observations in the present study may indicate that *Simarouba glauca* act as a protective agent against gentamicin induced Nephrotoxicity. However further research are needed to elucidate correct mechanism of action of Nephroprotective activity of *Simarouba glauca.*

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