



PROTECTIVE EFFECT OF AQUEOUS AND METHANOLIC EXTRACTS OF *LAGENARIA SICERARIA* SEEDS IN GENTAMICIN INDUCED NEPHROTOXICITY

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ABSTRACT

The aim of present study was to carry out the preliminary phytochemical studies and nephroprotective activity of methanolic and aqueous extracts of *Lagenaria siceraria* seeds, family cucurbitaceae. These studies revealed the presence of flavonoids, tannins (ellagitannins), saponins, polyphenols, triterpenes, lagenin (protein) in the extracts. The extract was found to be potent diuretic which causes excretion of sodium and potassium. Gentamicin is an extensively used aminoglycoside antibiotic. It has been reported to produce nephrotoxicity even at normal therapeutic dose level. Gentamicin was administered intraperitoneally at a dose of 80mg/kg body weight for 9 days. The biochemical parameters viz. serum creatinine, blood urea nitrogen (BUN) and serum uric acid was found to be significantly increased whereas serum total protein was decreased. Histopathological sections showed marked glomerular, peritubular and blood vessel congestion. These increased levels of biochemical parameters and extent of renal damage were decreased by the methanolic and aqueous extracts of *Lagenaria siceraria* seeds at a dose of 250mg/kg, Cystone tab. (500mg/kg) was used as reference standard to compare with the toxicant and test group animals.

Keywords: *Lagenaria siceraria*, nephroprotective activity, gentamicin, cystone tab.

INTRODUCTION

The plant *Legenaria siceraria* (cucurbitaceae) commonly known as "Bottle gourd" is a common fruit vegetable used in India. It is widely used as folkloric remedies in indigestion, constipation, liver complications and urinary disorders etc¹. Its aerial part, seeds, fruit are used for their medicinal properties since time immemorial. The fruit is also used as diuretic, cardiogenic, cardioprotective and nutritive agent. The fruit is also reported to be a good source of vitamin B complex and choline along with fair source of vitamin C and Beta-carotene. It is also reported to contain cucurbitacins, flavonoids, polyphenols, fibres and ellagitannin.²⁻⁵ The *Lagenaria siceraria* fruit is reported to possess antioxidant activity,⁶ hypolipidemic and antihyperlipidemic effects in normocholesterolemic and triton-induced hyperlipidemic rats.⁷ HPLC analysis of methanol extract from plant shows the presence of flavon-C glycosides.⁸ Lagenin, a novel protein has been isolated from lyophilized extract of seeds.⁹ *Lagenaria.siceraria* possesses immunoprotective, antitumor, anti HIV and antiproliferative properties.

Free radicals are highly reactive substances formed in the body as a result of metabolic processes. Many of these molecular species are oxygen (and sometimes nitrogen) centered free radicals and its non radical products.¹⁰ The term "reactive oxygen species" (ROS) collectively denotes oxygen centered radicals (super oxide and hydrogen radicals) as well as non-radical species derived from oxygen such as hydrogen peroxide (H₂O₂), singlet oxygen (O₂) and hypochlorous (HOCL) acid. The increased production of reactive oxygen species seems to accompany most forms of tissue injury. Free radicals can also react with DNA, protein or lipids in the cell membrane and cause damages.¹¹ The involvement of reactive oxygen species in aging and in many chronic diseases has been considered the defense provided by antioxidant systems is crucial for the survival of

organisms. Detoxification of reactive oxygen species in the cell provided by both enzymatic and nonenzymatic systems which constitute the antioxidant defense system. These antioxidants play a role in delaying, intercepting or preventing oxidative reactions catalyzed by free radicals.¹²

Aminoglycosides have long been one of the common causes of drug induced-nephrotoxicity. Gentamicin is a very effective antibiotic in treating gram-negative bacterial infection in both humans and animals. Gentamicin induced nephrotoxicity is a models of acute renal failure caused by oxidative stress generated through the induction of superoxide.¹³ It has been demonstrated that getamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules. It is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis followed by deterioration and renal failure.¹⁴ The toxicity of gentamicin is believed to relate to generation of reactive oxygen species in kidney. Several reports have documented the pathogenesis of aminoglycosides-induced renal tubular cell injury such as derangement of lysosomal, mitochondrial and plasma membrane structure. Furthermore results of many studies have been shown that the altered concentrations of various biochemical indicators of oxidative stress in kidney tissue are due to gentamicin because of the obvious mediation of reactive oxygen species in gentamicin induced renal damage, several antioxidant agents have been used to block gentamicin induced- nephrototoxicity.¹⁵⁻¹⁶

Extensive pharmacological evaluation has been done using *Lagenaria siceraria* seeds. The present study was carried out to evaluate nephroprotective activity of methanolic and aqueous extracts of *Lagenaria siceraria* seeds in rats.

MATERIALS AND METHODS

Cystone tablet was purchased from local market, Gulbarga. The diagnostic kits were purchased from Pathozyme, manufactured by Trans Asia biomedical Ltd. Baddi, Dist. Solan (H.P), India. The dried seeds of *Lagenaria siceraria* were purchased from M/s. Monarch Agro Genetics, Hyderabad. Albino Wistar rats of either sex weighing between 180-220g were selected for the present experimental study (n=6 in each group). Animals were acclimatized for one week to laboratory conditions (Temperature, 23±2°C, humidity 50 ±5%, and 12-hour light dark cycle) before study. The animal care and handling was carried out according to the guidelines set by CPCSEA. Animals were provided free access to food with water *ad libitum*. Animal studies were performed with prior permission of institutional animal ethics committee (IAEC) of HKES College of Pharmacy, Gulbarga (Protocol No. HKE COP/IAEC/31/ 2010-11/ CPCSEA).

Extraction of Plant Material

Methanolic extract

Powdered crude drug (250g of the fresh air-dried) of *Lagenaria siceraria* seeds were extracted successively with petroleum ether 60-80% first to de-fat (as the seeds consists of high percentage of oil, fats). Again the final product (extract) was extracted with methanol (60-80%) in soxhlet extraction apparatus for about 36 hours. The colorless solvent in the siphon tube was taken as the termination of extraction. The methanolic extract was air dried at room temperature, weighed and percentage yield was calculated. Extract was preserved in a refrigerator till future use. Preliminary phytochemical analysis was carried out by different methods of phytochemical analysis. The percentage yield of methanolic extract obtained was 13.75%.

Aqueous extract

Powdered crude air-dried drug of *Lagenaria siceraria* seeds were extracted by the process of maceration for one week. Drug powder along with distilled water in a ratio of 1:3 followed by 1 to 2 ml chloroform was soaked and stirred at regular intervals and was kept in a vessel for one week. The chloroform was used as a preservative to avoid fungal growth. On day eight, this was filtered through Whatmann's filter paper and the final extract was evaporated to dryness on a water bath at 50-70°C. It was stored in a refrigerator till future use; the percentage yield was 7.85%. A known volume of extract was suspended in distilled water and was orally administered to the animals by gastric intubation using a force feeding needle during the experimental period.

Preparation of dose for test dried extract and standard drugs

Seed extract (250mg/kg b.w) were formulated as suspension in distilled water using gum acacia as suspending agent. The strength of the suspension was according to the dose administered and was expressed as weight of dried extract.

Cystone (500mg/kg b.w) was used as the reference standard drug for evaluating the nephroprotective activity which was made into suspension in distilled water using gum acacia.

Acute Toxicity Studies

Lagenaria siceraria is being used since ancient time as a vegetable and fresh juice obtained from 200-300gm of fruit is recommended in 'Dudhi therapy'.¹⁷ According to earlier reports the dose of *Lagenaria siceraria* seed extract 250mg/kg p.o was used as hepatoprotective¹⁸. Therefore the above dose was considered as non-toxic and hence used in same dosage in this study.

Gentamicin induced Nephrotoxicity in rats

Albino rats (180-220) of either sex were used for the study; animals were divided in to four groups.¹⁹

Group-I served as normal control group and received distilled water p.o for eight days. Group II served as a toxicant, gentamicin to induce nephrosis. The gentamicin treated group received 80mg/kg/day by the intraperitoneal (IP) route for 8 days. Group III-received gentamicin with cystone 500mg/kg/day/oral as a reference standard for 8 days. Group IV-received gentamicin followed by aqueous extract of *Lagenaria siceraria* seeds (250/kg) for 8 days. Group V-received gentamicin followed by methanolic extract of *Lagenaria siceraria* seeds (250mg/kg) for 8 days. On the 9th day blood samples were collected via retro orbital puncture in overnight fasted animals under mild ether anesthesia. The collected samples were allowed to clot and serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for serum creatinine, blood urea nitrogen (BUN), serum uric acid and serum total protein by using kits. Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination.

Statistical Analysis

The results of the study were expressed as standard error of mean ± SEM (n=6), data was analyzed by using one way analysis of variance (ANOVA) followed by Dunnett 't' test for multiple comparisons. Values with (***)P<0.001 were considered to be statistically significant.

RESULTS

The histopathology of the kidney revealed that there was cortical congestion, inflammation, number of glomeruli reduced in gentamicin group. The present study showed that gentamicin induces renal injury as evidenced by decrease renal function in experimental animals. The study reveal that administration of gentamicin at 80mg /kg body weight produced a significant increased in blood urea nitrogen (BUN), serum creatinine and serum uric acid while the serum total protein level decreased viz. 54.20, 1.90, 7.07, 3.50 units followed by significant decrease in the weight of kidney of the experimental animals. The pretreatment with aqueous extract of *Lagenaria siceraria* at the dose level of 250 mg/kg. viz. 28.03, 0.61, 2.63, 5.40 units and alcoholic extract of *Lagenaria siceraria* viz. 29.55, 0.64, 2.67, 5.49 was found to be reverse the serum biochemical parameters.

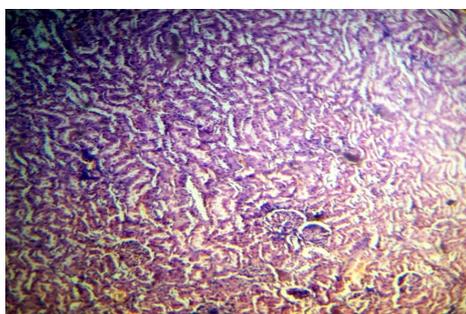
Control animals, showed normal glomerular and tubular histology, concurrent treatment with the aqueous and methanolic extracts was found to reduce such changes in kidney histology induced by gentamicin treatment .According to the pathological result it can be inferred that extracts of *Legenanaria siceraria* had protective effect against degenerative injury caused by gentamicin.

Table 1: Influence of methanolic and aqueous extracts of *Lagenaria siceraria* seeds on physical and biochemical parameters against gentamicin-induced nephrotoxicity in rats

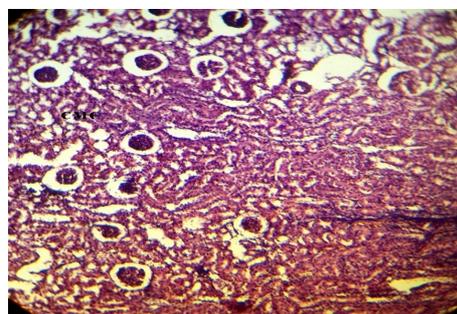
Group	Treatment	Dose	Physical parameter	Biochemical parameters			
			Body wt. (% change)	Total Protein (g/dl)	Serum Creatinine (mg/dl)	BUN (mg/dl)	Serum Uric acid (mg/dL)
I	Normal Control	Equivalent volumes	8.95±0.56	5.23±0.06	0.54±0.02	23.73±1.59	2.64±0.20
II	Gentamicin (Toxic Control)	80mg/kg	-15.85±0.68***	3.50±0.14***	1.90±0.03***	54.20±3.93***	7.07±0.18***
III	GENT + CYST (Standard)	80mg/kg + 500mg/kg	4.11±0.55***	5.57±0.16***	0.67±0.02***	26.9±2.26***	2.80±0.11***
IV	GENT+AELS	80mg/kg + 250mg/kg	5.37±0.28***	5.40±0.13***	0.61±0.03***	28.03±1.50***	2.63±0.13***
V	GENT+MELS	80mg/kg + 250mg/kg	3.31 ±0.32***	5.49±0.18***	0.64±0.03***	29.55±1.69***	2.67±0.12***

N=6, P values: ***P<0.001, when compared to group II vs. group IV and group II vs. V, one-way analysis of variance (ANOVA) followed by Dunnett 't' test. GENT-Gentamicin, CYST-Cystone tablet, AELS-Aqueous extract of *Lagenaria Siceraria*, MELS- Methanolic extract of *Lagenaria Siceraria*

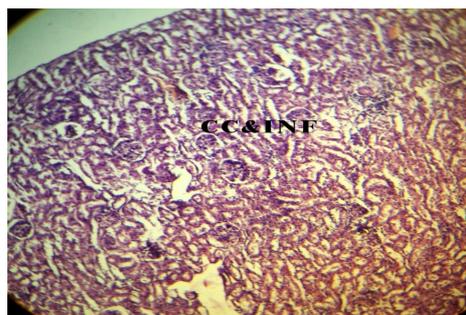
Histopathological examinations of rat kidney



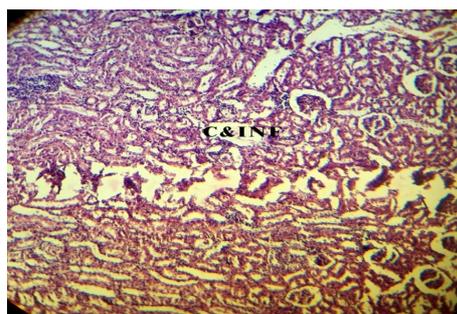
a. Group 1 (normal control)



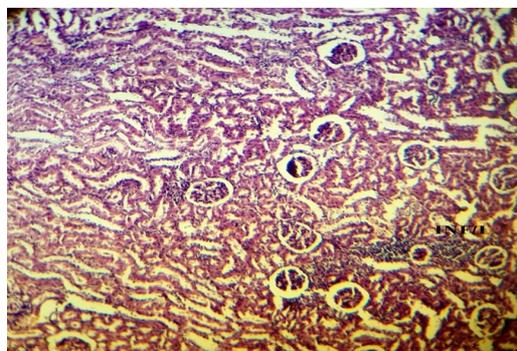
c. Group 3 (standard): CMC-Cortical mild congestion in both glomerular and peritubular



b. Group 2 (positive control): CC-Cortical glomerular and peritubular Congestion, INF-Interstitial inflammation



d. Group 4 (AELS): C & INF-Cortical very mild congestion and inflammation



e. Group 5 (MELS): INF-mild interstitial inflammation

DISCUSSION

Preliminary phytochemical screening of the aqueous and methanolic extracts showed the presence of flavonoids, phenols, carbohydrates, steroids, tannins, glycosides, terpenes and saponins.

In the present study, gentamicin induced kidney damage and its recovery by herbal drugs were assessed by alteration in the clinically important biochemical variables such as serum creatinine, blood urea nitrogen (BUN), serum uric acid which were found to be significantly (**P<0.001) increased in rats treated with gentamicin, while serum total proteins level was decreased. Whereas treatment with the aqueous and methanolic extracts of *Lagenaria siceraria* seeds reversed the effect of gentamicin indicating nephroprotective activity. There is simultaneous significant decreased in the gentamicin-induced nephrotoxicity when the antioxidant defense system is effective. The increased production of reactive oxygen species in gentamicin-induced nephrotoxicity may be a result of inactivation of antioxidant enzymes such as superoxide dismutase and GSH-Px. A relationship between nephrotoxicity and oxidative stress has been confirmed by many investigators. The impairment in kidney functions is accompanied by increase in serum creatinine and urea level and kidney tissue MDA levels that indicates lipids peroxidation. It is one the essential compounds for maintaining cell integrity participation in the cell metabolism²⁰⁻²². The significant and progressive weight loss in gentamicin treated rats may possible be due to the injury of renal tubules and the subsequent loss of the tubular cell to reabsorb water, leading to dehydration and loss of body weight. Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidation chain reaction small concentration and thereby eliminate the threat of pathological process. Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidant activity. They show antiatherogenic and anticarcinogenic activities by blocking LDL oxidation of carcinogens. Ellagitannins from *Lagenaria siceraria* have been shown to decrease the lipid peroxide formation and restoration of glutathione status and the activities of antioxidant enzymes during gentamicin-induced nephrotoxicity. *Lagenaria siceraria* might have exhibited nephroprotective activity by the virtue of its antioxidant activity.

CONCLUSION

The present study revealed that aqueous and methanolic extracts of *Lagenaria siceraria* is a good source of phytochemicals with antioxidant properties. The extracts also reverses the nephrotoxicity induced by gentamicin.

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