Evaluation of Nephroprotective Activity of Shilajit in Gentamicin induced Nephrotoxicity

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Abstract :

Gentamicin is an aminoglycosidal antibiotic widely used in treating severe gram negative infections. However its limited uses due to renal dysfunction. The present investigation was carried out to evaluate the nephroprotective activity of Shilajit in gentamicin induced nephrotoxicity. Shilajit (200, 400 and 800 mg/kg p.o.) was administrated for 8 days. Gentamicin was administrated at the dose of 80 mg/kg i.p. daily from 4th to 10th day. Gentamicin (alone) treated group showed increased levels of urine and serum urea, creatinine, uric acid, potassium and decreased levels of urine and serum sodium levels, which were significantly retrieved in group pretreated with Shilajit (400 and 800 mg/kg p.o.), where as Shilajit (200 mg/kg p.o.) showed insignificant results. The antioxidant activity of Shilajit revealed that the level of urine and serum in Shilajit pretreated group when compared with gentamicin alone treated group. The histopathological analysis also showed the protective nature of Shilajit in Gentamicin induced renal damage. The main goal of this paper is to provide an actualized and mechanistic vision of pathways involved in glomerular toxic effects of Gentamicin.

Keywords : Shilajit, Gentamicin, Nephrotoxicity.

Introduction : Kidney is the primary organ of the urinary system, which purifies the blood by excreting wastes products and toxic materials from the body through urine. It is one of the vital organs of the body because it helps in maintaining the fluid homeostasis, electrolyte balance, blood pressure, etc [1].

Nephrotoxicity occurs when the renal blood is exposed to a nephrotoxic drug or toxin that causes damage to the kidneys. When kidney damage occurs, body is unable to rid of excess urine and wastes from the body and blood electrolytes (such as potassium and magnesium) will all become elevated[2,3]. Number of chemicals and therapeutic agents are associated with the development of renal failure. One of them is gentamicin. It is an aminoglycoside antibiotic, widely used in the treatment of Gram-negative infections. Gentamicin causes the major adverse effects of nephrotoxicity and ototoxicity; this is being continuously used in clinical practice due to its high bactericidal efficacy, low-cost and limited bacterial resistance[4]. Gentamicin induces lysosomal phospholipidosis that disrupts normal renal function[5]. Aminoglycoside induced nephrotoxicity is characterized by slow rise in serum creatinine, tubular necrosis and marked decrease in glomerular filtration rate and in the ultra filtration coefficient[6]. There are several mechanisms have been suggested by various researches regarding gentamicin induced nephrotoxicity. One of the mechanisms is supposed to be related with generation of reactive oxygen species (ROS) in kidney[7].

According to the World Health Organization (WHO), traditional medicine (TM) incorporates health practices, approaches and knowledge of plant, mineral and animalbased medicines, applied singularly or in combination to treat and prevent illnesses or maintain well-being[1,2]. Shilajit is one of the herbomineral, which is having fulvic acid and humic acid like active constituents. These components have antioxidant activity which may exhibit good nephro-protection against gentamicin induced nephrotoxicity [8,9]. Shilajit has been used as a rejuvenator and an adaptogen for thousands of years, in one form or another, as part of traditional systems of medicine and hence the current study was planned.

Materials and Methods:

Animals : The healthy Wister albino rats of either sex weighing between 180 – 250 g were taken for the study. They were housed under controlled conditions of temperature (23±2°c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the CPCSEA guidelines.

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Drug & Chemicals : Shilajit was purchased from Patanjali, Gentamicin was purchased from Cipla and other reagents were purchased from local market. All chemicals used in these studies were of analytical grade.

Preparation and dosing schedule : [10,11]

The selected doses of Shilajit 200, 400 and 800 mg/kg

b.w. for the rats. They are given by oral route using oral gavage. Standard Cystone 500 mg/kg b.w. was used.

Experimental design: [12]

The animals were kept for acclimatization and then they were divided randomly into 6 groups, each consisting of 6 rats. Group I (vehicle control) was administered distilled water throughout the course. To the group II (Gentamicin treated group) was administered at a dose of 80 mg/kg i.p. for eight days for the induction of nephrotoxicity. For the first three days these animals are treated with normal saline. Three days before the administration of gentamicin, Cystone 500 mg/kg p.o. and Shilajit at a dose of 200,400 and 800 mg/kg. b.w. were administered to group III, IV, V, and VI respectively and this treatment was continued for eight days together with gentamicin administration. The normal rats were administered with vehicle alone throughout the course.

Table No. 1 :
Gentamicin induced nephrotoxicity medel

Sr. No.	Groups n=6	Treatment and dose/ day	Observation
1	Group I	Vehicle control (1 ml distilled water) 0 to 10th day	a) Morphological study on 1st and 11th day
2	Group II	Negative control Gentamicin (80 mg/kg i.p.) 4th to 10th day	b)Serum Analysis on 11th day
3	Group III	Gentamicin (80 mg/kg i.p.) 4th to 10th day + Standard Cystone (500 mg/kg p.o.) 1st to 10th day	c)Urine analysis on 1st and 11th day
4	Group IV	Gentamicin (80 mg/kg i.p.) 4th to 10th day + Shilajit dose (200 mg/kg p.o.) 1st to 10th day	d) Kidney damage on 11th day
5	Group V	Gentamicin (80 mg/kg i.p.) 4th to 10th day + Shilajit dose (400 mg/kg p.o.) 1st to 10th day	
6.	Group VI	Gentamicin (80 mg/kg i.p.) 4th to 10th day +Shilajit dose (800 mg/kg p.o.) 1st to 10th day	

Collection and analysis of urine : On the 1st and 10th day all aminals are separated out in metabolic cages and the urine were collected after 24 hours. From the collected urine samples of rats, urinary sodium, potassium,creatinine, uric acid and urea were estimated using autoanalyzer and diagnostic kits.

Collection of serum : On the 11th day all animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro orbital plexus by using fine glass capillary and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 3000 rpm for 10 min. The separated serum was used for estimation of Creatinine, Urea, Uric acid, Sodium and Potassium.

Histopathological investigations : On the 11th day the animals were sacrificed and abdomen was cut open, the kidney was dissected out. The kidneys were sectioned longitudinally into two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. These sections were stained with hemotoxylin and eosin and were observed under a computerized light microscope (magnification power-40X). [13]

Statistical analysis : Arithmetic means of the values of readings were calculated for each experiment the result obtained were used for statistical analysis using INTA software. The data obtained from various models of nephrotoxicity in rats experiments were subjected to analysis of variance (ANOVA) followed by Dunnetts t-test using INTA software. Value of $p < 0.01$ was considered statistically significant.

Results:

Morphological study : There was significant ($p<0.01$) decrease in the body weight and significant ($p<0.01$) increase in the kidney weight of Gentamicin administered group was observed when compared to normal group, whereas there was significant ($p<0.01$) increase in the body weight and significant ($p<0.01$) decrease in kidney weight was observed in the standard (Cystone 500 mg/kg p.o.) and Shilajit treated groups (400 & 800 mg/kg p.o.) when compared to gentamicin administered group. There was insignificant ($P>0.05$) increase in body weight and insignificant ($P>0.05$) decrease in kidney weight in the Shilajit (200 mg/kg p.o.) treated groups when compared to Gentamicin administered group.

Table No. 1: Effect of Shilajit on Body weight and kidney weight in Gentamicin induced nephrotoxicity.

Group No.	Treatment	Body Weight (gm)	Kidney Weight (gm)
1	Vehicle	217.50±1.78	1.142±0.03
2	Gentamicin	205.33±1.40##	1.510±0.03##
3	Gentamicin + Cystone	215.67±1.83**	1.198±0.02**
4	Gentamicin +Shilajit 200	208.17±1.01ns	1.472±0.01ns
5	Gentamicin + Shilajit 400	213.00±1.09**	1.378±0.01**
6	Gentamicin + Shilajit 800	216.83±2.00**	1.207±0.01**

N=6, values are expressed as Mean ± SEM. Comparision were made as follows, # p < 0.05, ## p < 0.01 when compared with normal control. * p <0.05, ** p < 0.01 when compared with negative control. (values are compared on 11th day by one way A NOVA Dunnett t test) N.S. – nonsignificant.

Urine Analysis : There was significant (P<0.01) increase in urine levels of urea, creatinine, uric acid, potassium and significant (P<0.01) decrease in urine volume and urine sodium levels in gentamicin administered group when compared to normal group. Whereas there is significant (P<0.01) decrease in urine levels of urea, creatinine, uric acid, potassium and significant (P<0.01) increase in urine volume and urine sodium in Cystone (standard) administered group when compared to gentamicin administered group.

There was insignificant (P>0.05) decrease in urine levels of urea, creatinine, uric acid, potassium and insignificant (P>0.05) increase in urine volume and urine sodium levels in Shilajit (200 mg/kg p.o.) treated group when compared to gentamicin administered group. Whereas there is significant (P<0.01) decrease in urine levels of urea, creatinine, uric acid, potassium and significant (P<0.01) increase in urine volume and urine sodium in Shilajit (400 and 800 mg/kg p.o.) administered group when compared to gentamicin administered group.

Table No. 2: Effect of Shilajit on urine parameters in Gentamicin induced nephrotoxicity.

Group No.	Treatment	Urine Volume (ml/day	Urine urea (mg/dl)	Urine creatinine (mg/dl)	Urine uric acid (mg/dl)	Urinary Na+ (meq/L)
1	Vehicle	2.325±0.04	35.528±0.71	1.361±0.09	4.498±0.14	142.76±1.55
2	Gentamicin	2.000±0.05##	56.337±1.12##	3.018±0.16##	9.884±0.16##	124.88±1.35##
3	Gentamicin+Cystone	2.215±0.03**	39.894±1.80**	1.514±0.14**	5.227±0.15**	140.67±1.43**
4	Gentamicin+Shilajit 200	2.017±0.03ns	55.833±1.31ns	2.892±0.12ns	9.145±0.19*	127.79±1.33ns
5	Gentamicin+Shilajit 400	2.173±0.02**	49.382±1.32**	2.281±0.19**	9.000±0.19**	131.82±0.98**
6	Gentamicin+Shilajit800	2.213±0.03**	40.517±1.15**	1.708±0.12**	5.122±0.09**	139.98±1.15**

N=6, values are expressed as Mean ± SEM. Comparision were made as follows, # p< 0.05, ## p< 0.01 when compared with normal control. * p<0.05, ** p< 0.01 when compared with negative control. (values are compared on 11th day by one way ANOVA Dunnett t test) N.S. – nonsignificant.

Serum Analysis : There was significant (P<0.01) increase in serum urea, serum creatinine, serum uric acid, serum potassium and significant (P<0.01) decrease in serum sodium levels in gentamicin administered group whem compared to normal group. Whereas there was significant (P<0.01) decrease in serum levels of urea, creatinine, uric acid, potassium and significant (P<0.01) increase in levels of serum sodium in Cystone standard administered group when compared to gentamicin administered group.

There was insignificant (P>0.05) decrease in serum level of urea, creatinine, uric acid, potassium and insignificant (P>0.05) increase in serum sodium levels in Shilajit 200 mg/kg p.o. when compared to gentamicin administered group. Whereas there was significant (P<0.01) decrease in serum levels of urea, creatinine, uric acid, potassium and significant (P<0.01) increase in level of serum sodium in Shilajit (400 and 800 mg/kg p.o.) administered group when compared to gentamicin administered group.

Table No. 3: Effect of Shilajit on serum parameters in Gentamicin induced nephrotoxicity.

Group No.	Treatment	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)	Serum uric acid (mg/dl)	Na+ (meq/L)	K+ (meq/L)
1	Vehicle	37.427±1.25	1.047±0.11	2.547±0.01	146.38±0.48	4.923±0.01145
2	Gentamicin	56.329±1.22##	1.954±0.12##	5.942±0.01##	133.19±0.71##	5.847±0.02##
3	Gentamicin+ Cystone	39.049±1.44**	1.154±0.11**	3.042±0.01**	143.07±0.185**	5.095±0.01**
4	Gentamicin+Shilajit200	54.779±1.30ns	1.871±0.12ns	5.148±0.01ns	134.95±0.42*	5.735±0.01ns
5	Gentamicin+Shilajit400	48.595±1.43**	1.372±0.12**	4.637±0.01**	135.53±0.34**	5.558±0.01**
6	Gentamicin+Shilajit800	40.948±1.54**	1.099±0.11**	4.435±0.02**	142.20±0.25**	5.190±0.01**

N=6, values are expressed as Mean ± SEM. Comparision were made as follows, # p<0.05, ##p< 0.01 when compared with normal control. * p<0.05, **p< 0.01 when compared with negative control. (values are compared on 11th day by one way ANOVA Dunnett t test) N.S. – nonsignificant.

Histopathology:

Table No. 4: Results of the histopathological examination in gentamicin induced nephrotoxicity

Group No.	Treatment	Glomerular congestion	Glomerular hemo-rrhage	Tubular Dilatation	Cast in tubules	Interstitial Inflam-mation	Blood vessels	Necrosis
1	Vehicle	Absent	Absent	Absent	Absent	Absent	Normal	Absent
2	Gentamicin	Present	Present	Present	Present	Present	Conge-sted	Present
3	Gentamicin+Cystone	Absent	Absent	Present	Present	Absent	Normal	Absent
4	Gentamicin+Shilajit200	Present	Present	Present	Present	Present	Conge-sted	Present
5	Gentamicin+Shilajit400	Absent	Absent	Present	Absent	Present	Normal	Absent
6	Gentamicin+Shilajit800	Absent	Absent	Absent	Absent	Absent	Normal	Absent

Histopatholgical observations:

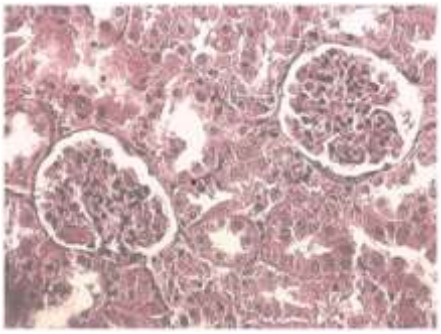

Fig 1. Group I : Normal (x40) Showing normal glomeruli. necrosis, cast in tubules etc.

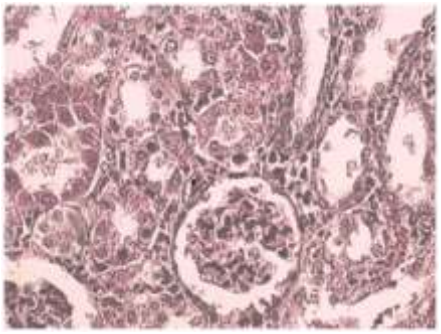

Fig 2. Group II: Negative control (Gentamicin) (x40) Showing dilated tubules, glomerular hemorrhage,

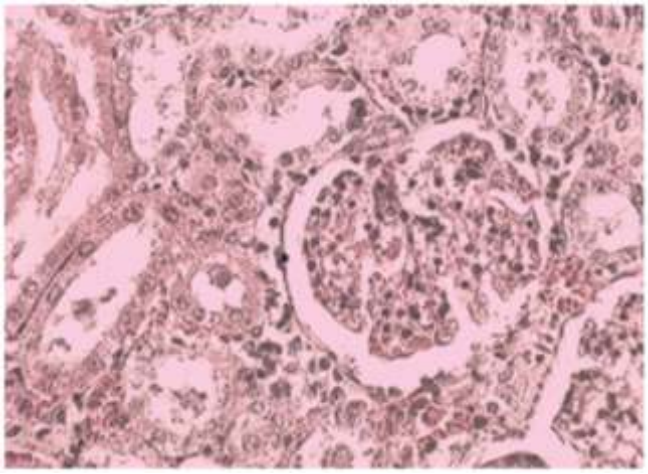

Fig.3 Group III: positive control [Gentamicin + Cystone] (x40) Showing dilated tubules and few cast in Tubule.

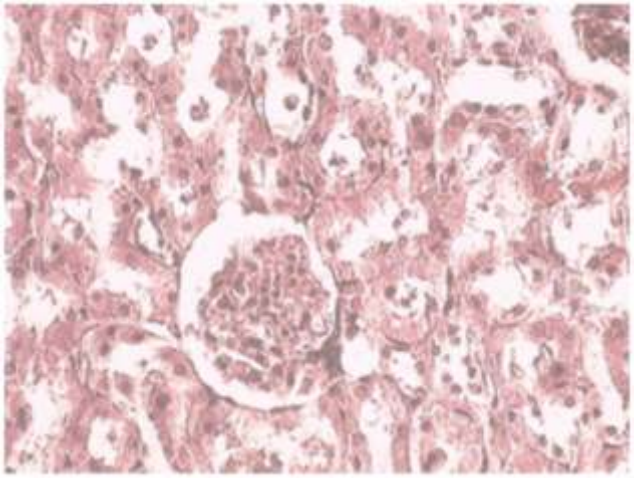

Fig. 4 Group IV: [Gentamicin+Shilajit200] (x40) Showing glomerular hemorrhage, disruption of glomerular, cast in tubules & congested vessels.

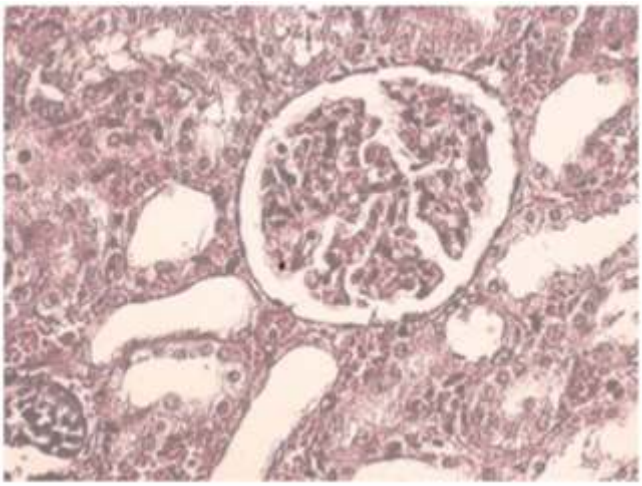

Fig 5 Group V: [Gentamicin+Shilajit400] (x40) Showing glomerular hemorrhage, and few

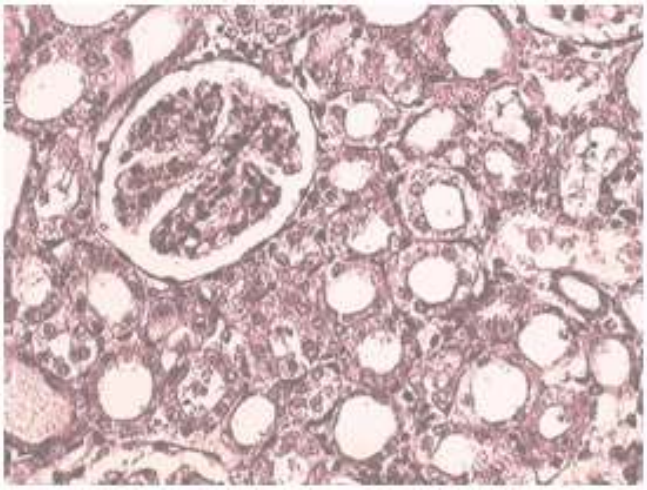

Fig. 6 Group VI: [Gentamicin+Shilajit800] (x40) Showing normal glomeruli, absence of tubular cast, necrosis, etc.

Discussion : Gentamicin induced nephrotoxicity is a model of acute renal failure caused by oxidative stress generated through the induction of superoxide anion. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules [4].

The significant and progressive weight loss in gentamycin treated rats may possibly be due to the injury of renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight [5]. Shilajit (400 mg/kg and 800 mg/kg p.o.) showed dose dependant increase in body weight and decrease in kidney weight suggesting its possible involvement in cells regeneration inside the body thus improving the condition of the internal organs[8].

Results showed that Shilajit (400 mg/kg and 800 mg/kg p.o.) significantly normalized biomarkers levels, suggesting its possible role in nephrotoxicity.Shilajit (400 mg/kg and 800 mg/kg p.o.) showed dose dependant increase in body weight, urine sodium and serum sodium levels and decrease in kidney weight, urine and serum urea, creatinine, uric acid and potassium level; suggesting its possible involvement by inhibiting oxidative stress and regeneration in cells [9]. The histopathological changes induced by gentamicin administration significantly protected by pretreatment with Shilajit (400 mg/kg and 800 mg/kg p.o.) but Shilajit (200 mg/kg p.o.) treated group gave negative results, which correlates with biochemical parameters. Hence it is suggested that Shilajit is potentially useful for the prevention of renal toxicity

during gentamicin chemotherapy.

No significant decrease or increase was found in biochemical parameters in the test drug treated animals compared with control animals. Therefore, it is clear that there is no any adverse effect in kidney due to use of Shilajit. These results suggest that Shilajit has an ability to prevent gentamicin-induced nephrotoxicity in albino rats.

Conclusion : The toxicity of gentamicin is believed to relate with generation of reactive oxygen species (ROS) in kidney and administration of antioxidants have proved marked protection against gentamicin-induced impairment of renal function. Hence, it may be concluded that Shilajit showed nephroprotective action against gentamicin nephrotoxicity possibly through its antioxidant property. The chemical constituent present in Shilajit are Fulvic acid, Humic acid, etc. Which acts as a adoptogen and antistress agent [8]. This is further supported by the results of histopathological and biochemical parameters of this study.

Further investigations on the mechanism of action of Shilajit are required and may have a considerable impact on future clinical treatments of patients with renal failure.

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