

AN INVESTIGATION OF THE NEPHROPROTECTIVE EFFECTS OF TRIPHALA-AN AYURVEDIC FORMULATION IN EXPERIMENTAL MODELS OF NEPHROTOXICITY.

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ABSTRACT

*The objective of this study was to evaluate the nephroprotective activity of triphala on gentamicin induced nephrotoxicity in albino wistar rats. Twenty- four albino wistar rats were divided into four groups (n=6). Rats in group I (normal) received normal saline (p.o.) per day for 11 days. The group II rats were treated with gentamicin (80mg/kg,i.p.) for 8 days, in order to induce nephrotoxicity. The rats in group III and IV were pre treated with triphala (500mg/kg, p.o. and 1000 mg/kg, p.o.) for first three days followed by gentamicin (80mg/kg, i.p.) treatment till eleventh day. Body weight, urinary glucose, sodium, potassium and creatinine levels, serum creatinine and blood urea nitrogen (BUN) levels, kidney weight and lipid peroxidation were evaluated along with histopathological investigation in various experimental groups of rats. It was observed that the gentamicin treated rats (group II) had significantly reduced (***) P <0.001) body weight, urinary sodium, potassium and creatinine while significantly elevated values (***) P <0.001) of urinary glucose, serum urea (BUN) and creatinine, kidney weight and lipid peroxidation when compared to group I (normal) rats. Histological observations of rat kidney tissues further correlated the nephroprotective effect of triphala. The result of this study provides the experimental evidence for claiming the nephroprotective effect of triphala.*

Keywords: Blood urea nitrogen, gentamicin, lipid peroxidation, nephrotoxicity.

INTRODUCTION

Nephrotoxic injury is damage to one or both of the kidneys that results from exposure to a toxic material, usually through ingestion. Nephrotoxic injury can lead to acute renal failure, in which the kidneys suddenly lose their ability to function, or chronic renal failure, in which kidney function slowly deteriorates. If unchecked, renal failure can result in death. Chronic exposure to drugs, occupational hazards, or environmental toxins can lead to chronic interstitial renal diseases.¹ Aminoglycoside gentamicin sulphate (GS) is commonly used for the treatment of Gram-negative bacterial infection, but nearly 13-30% patients suffer from nephrotoxicity thus limiting its use. Gentamicin Sulfate has been used clinically due to its wide spectrum of activities against Gram-negative bacterial infection caused by pseudomonas, proteus and serratia. Gentamicin induced nephrotoxicity is characterized by an increase in generation of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals, hydrogen peroxide and reactive nitrogen species (RNS) in kidneys and lead to renal injuries. GS induced renal damage is linked with lipid and protein oxidation in renal cortex .GS induces poly (ADP-ribose) polymerase in proximal tubules. GS is not metabolized in the body but is essentially eliminated by glomerular filtration and partially reabsorbed by proximal tubular cells. The specificity of gentamicin induced nephrotoxicity is apparently related to its accumulation in the renal proximal convoluted tubules causing a number of morphologic, metabolic and functional alterations.^{2,3}

Triphala (in Sanskrit tri = three and phala = fruits) is composed of the three myrobalans, *Terminalia chebula* (Haritaki), *Terminalia belerica* (Bibhitaki), and *Emblica officinalis* (Amalaki) and is one of the most commonly used Ayurvedic preparations. The formulation generally consists of equal proportions of pericarps of these myrobalans. Triphala has been described in the ancient Ayurvedic text as a Tridoshic rasayana, a therapeutic agent with balancing and rejuvenating effects on the three humours or constitutional elements in Ayurveda vata, pitta and kapha. *T. chebula* and *T. belerica* have a warm energy, while *E. officinalis* is cool in nature. Triphala, being a combination of all three, is therefore balanced, making it useful as an internal cleansing, detoxifying formula. Triphala is regarded as an important rasayana and good purgative in Ayurvedic medicine. Recipe for this traditional herbal supplement is described in the traditional Indian texts, the Charak Samhita and Susruta Samhita which date back to 1500 B.C.^{4,5}

MATERIALS AND METHODS

Animals: Healthy albino wistar rats, weighing 200-250g, were purchased from authentic animal supplier in Bangalore, which were maintained in the animal house of PES college of Pharmacy, Bangalore for experimental purpose. All these animals were housed in temperature controlled room 25±1°C, relative humidity 45-55% and a 12 h light/ 12h dark cycle. They were acclimatized for 7 days and fed with standard rat chow (Amruth Animal Feeds Pvt Ltd, Bangalore, India) and water ad libitum. Animals were habituated to laboratory conditions for 48h prior to experiment, to minimize stress. All animal studies including protocols and experiments were performed in accordance to the guidelines and ethical principles as per the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA),⁶ Govt. of India, New Delhi. Prior approval was taken from the Institutional Animal Ethical Committee (IAEC approval No- **PESCP/IAEC/ 10 /11**) of P.E.S College of Pharmacy, Bangalore, Karnataka for conducting animal studies.

Chemicals: Triphala churna manufactured by Shree Baidyanath Ayurved bhawan PVT. LTD, Nagpur was purchased from local Baidyanath Pharmacy, Bangalore with batch no-115043. Each 10gram contains haritaki, bibhitaka and amalaki (weight-3.333g each) in ratio of 1:1:1 as per product literature The aqueous suspension of triphala was prepared by taking triphala powder in required quantity and to it distilled water was added (5g in 100ml) and homogenized to form uniform suspension of the extract. The extract was freshly prepared every day before dosing the animals.

Dosages: The dose of aqueous suspension of Baidyanath triphala powder for the pharmacological studies was selected on the basis of previous research work performed on triphala. The acute toxicity study was done as per the organization of economic co-operation and development (OECD) guidelines and it was found to be safe up to 2000 mg/kg. The dose of triphala was taken as 500mg/kg, b.w as low dose and 1000mg/kg, b.w. as high dose, based upon literature survey and previous work done.⁷

Grouping and dosage regimen: Twenty four albino wistar rats were divided into four groups with six animals each. (n=6)

Group I: Control which receives vehicle p.o. throughout the course.

Group II: Gentamicin (80 mg/kg/ i.p.) for 8 days.

Group III: Gentamicin (80mg/kg i.p) for eight days + aqueous solution of triphala (500mg/kg, p.o.) which is started 3 days prior to gentamicin treatment continued by 8 days gentamicin treatment

Group IV: Gentamicin (80mg/kg i.p) for eight days + aqueous solution of triphala (1000mg/kg, p.o.) which is started 3 days prior to gentamicin treatment continued by 8 days gentamicin treatment.⁸

Sample collection and biochemical assays: After the eleventh day treatment, all the rats were hydrated with the administration of additional vehicle (0.5ml/kg b.w) and were kept in individual metabolic cages for 24-hour urine collection. The collected urine was transferred to clean eppendorf tubes and centrifuged at 2000 r.p.m for 10 min. The supernatant was transferred to cleaned eppendorf tubes and used for estimation of sodium, potassium, glucose and creatinine. 24h after the 11th day treatment blood was collected from retro orbital sinus under ether anaesthesia in eppendorf tubes and serum was separated by cold centrifugation at 3000 r.p.m for 10 min. The supernatant serum was transferred to clean eppendorf tubes and used for the estimation of urea and creatinine. The serum and urinary estimations were carried out by using ERBA diagnostic kits.

Then, the rats were sacrificed by cervical dislocation method as per CPCSEA guidelines. Kidneys were isolated and weighed. They were thoroughly washed with ice- cold PBS solution. 10% homogenate was prepared in phosphate buffer saline (PBS) 0.05 M, pH 7) using a remi homogenizer at 25° C at 5000rpm for 15 min. The homogenate was centrifuged to remove unwanted cell debris, nuclei and mitochondria. It was used for estimation of kidney tissue anti oxidant levels.

Determination of lipid peroxidation (MDA) levels: Malondialdehyde (MDA) levels were determined by the method of Ohkawa.et.al.,. In this process, 0.2ml of kidney tissue homogenate, 0.2ml of 8.1% sodium dodecyl sulfate, 1.5ml of 20% acetic acid and 1.5ml of 0.8% TBA were added. The volume of the mixture was made up to 4ml with distilled water and then heated at 95°C in a water bath for 60min. After incubation the tubes were cooled to room temperature and the final volume was made upto 5ml with distilled water in each tube. Then, 5ml of n-butanol: pyridine mixture (15:1, v/v) was added and shaken vigorously for 2 min. After centrifugation at 4000rpm for 10 min, the upper organic layer was taken and its optical density (OD) was read at 532nm against blank. The levels of lipid peroxides were expressed as millimoles of thiobarbituric acid reactive substances (TBARS)/100g of cardiac tissue using an extinction co-efficient of $1.56 \times 10^5 \text{M}^{-1} \text{cm}^{-1}$.⁹

HISTOLOGICAL STUDIES

One kidney from each sacrificed rat was taken and fixed in 10% v/v neutral formalin and processed to paraffin wax. Sections (5 microns) were stained with haematoxyllin and eosin and were examined under light microscope at 40 X and 100 X magnifications.

STATISTICAL ANALYSIS

Statistical analysis was performed by one way Analysis of Variance (ANOVA) followed by Dunnet's test for post comparison using software Graph pad prism version 5.0. Results were expressed as mean \pm SEM. P values <0.05 were considered to be statistically significant.

RESULTS

There is a significant decrease in urinary sodium, potassium and creatinine levels while a significant increase is seen in urinary glucose levels in group II rats while compared to group I. The rats treated with triphala 500mg/kg, p.o. and 1000mg/kg, p.o. + gentamicin 80mg/kg,i.p. have showed significant nephroprotection by increasing urinary sodium, potassium and creatinine and by decreasing urinary glucose levels as showed in the table 1.

TABLE - 1 Showing the effect of vehicle, gentamicin and triphala on urinary sodium, urinary potassium, urinary glucose and urinary creatinine levels in rats

Group (n=6)	Treatment		Urinary sodium (mEq/day)	Urinary potassium (mEq/day)	Urinary glucose (mg/dl)	Urinary Creatinine (mg/dl)
	Dose and Route	Duration Days				
I	Vehicle, p.o.	11	7.195± 0.2343	15.133± 2.015	48.216± 1.18	6.505± 0.507
II	GEN (80mg/kg, i.p.)	7	3.932± 0.4287***	5.05± 1.126**	278.4± 35.03** *	1.360± 0.188***
III	Triphala (500mg/kg, p.o),	11	4.813± 0.1635***	13.216± 1.177**	129.317 ± 4.595*	4.923± 0.486***
	GEN (80mg/kg, i.p)	7				
IV	Triphala (1000mg/kg, p.o),	11	5.1± 0.5111**	16.166± 0.7633**	146.75± 7.215**	4.478± 0.414***
	GEN (80mg/kg, i.p)	7				

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ Control Vs treated groups using one way ANOVA followed by Dunnett's test.

TABLE – 2 Showing effect of vehicle, gentamicin and triphala on serum urea, serum creatinine, kidney weight and lipid peroxidation in rats.

Group (n=6)	Treatment		Serum urea (mg/dl)	Serum creatinine (mg/dl)	Kidney weight (g)	Lipid peroxidation
	Dose and Route	Duration				
I	Vehicle, P.O	11 days	21.35±0.7970	11.26±0.3663	0.8891 ± 0.0671	257.1±17.95
II	GEN (80mg/kg,.) i.p.	7 days	26.25±0.7584	22.28±1.327***	1.138±0.03233*	648.9±29.00***
III	Triphala (500mg/kg, p.o),	11days	19.41±0.2264	11.85±1.065***	1.017±0.004	483.3 ± 27.88**
	GEN (80mg/kg,.) i.p	7 days				
IV	Triphala (1000mg/kg, p.o),	11days	17.52±0.4424***	7.36±0.3303***	1.038 ± 0.0183	371.9±28.55***
	GEN (80mg/kg,.) i.p	7 days				

Values are expressed as mean ± S.E.M. n = 6. Significant values were compared with* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ Control Vs treated groups using one way ANOVA followed by Dunnett's test.

There is a significant increase in serum urea, serum creatinine, kidney weight and lipid peroxidation levels in group II rats while compared to group I. The rats treated with triphala (500mg/kg, p.o. and 1000mg/kg, p.o. + GEN 80mg/kg, i.p) have showed significant nephroprotection by decreased serum urea, creatinine and lipid peroxidation levels while compared to group II whereas the decrease in kidney weight in group III and IV was not found to be significant.

Histopathology of rat kidney

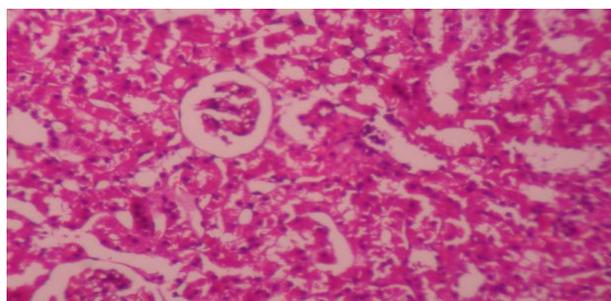


Fig I Effect of vehicle on rat kidney (X400)

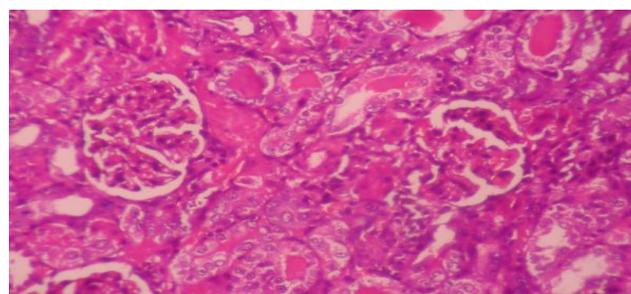


Fig II Effect of GM on rat kidney (X400)

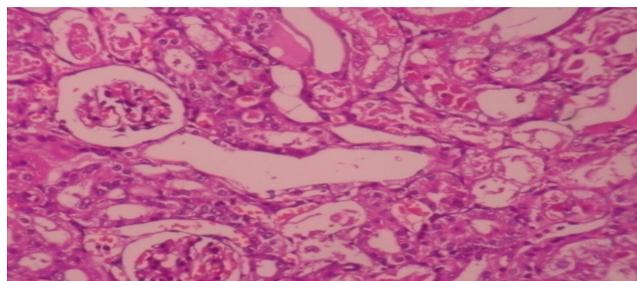
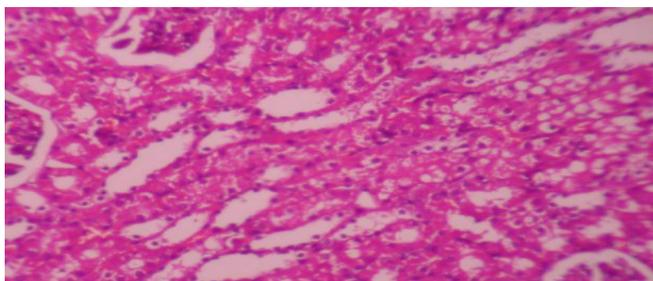


Fig III Effect of TRI 500 mg/kg + GEN on rat kidney(X400) Fig IV Effect of TRI 1000 mg/kg + GEN on rat kidney (X400)

Histopathological studies clearly indicate kidney damage in gentamicin only treated animals. In the present study, kidneys of group I (normal) rats show renal parenchyma of normal architecture. In group II (gentamicin treated) rats various degrees of focal lesions in many sections including tubular congestion, tubular desquamation and hyaline casts have been seen. The groups that received both triphala along with gentamicin respectively have shown mild glomerular necrosis, few intraluminal casts, moderate mononuclear inflammatory infiltration within the interstitium and blood vessels appearing unremarkable indicating the protective effect of triphala.

DISCUSSION

In the present study, the rats administered with gentamicin have shown significant decrease in the urinary sodium and potassium levels when compared to normal rats. As it is evident that the aminoglycosides also can induce renal potassium and magnesium wasting, with resulting hypokalemia and hypomagnesemia. Aminoglycosides produce tubular cell necrosis, which is largely confined to the proximal convoluted tubule and pars recta. Several tubular cell membrane functional changes occur with aminoglycoside exposure. The rats treated with triphala and gentamicin have shown significant increase in sodium and potassium levels when compared to gentamicin induced nephrotoxic rats suggesting the nephroprotection.¹⁰

There is significant decrease in the urine creatinine levels in gentamicin induced nephrotoxic rats when compared to normal rats. This is due to structural damage of the podocytes, pedicels of the visceral layer of the Bowman's capsule and the endothelium of the glomerular capillaries of the nephron. This disturbs the glomerular filtration of the creatinine. Hence creatinine excretion gets decreased and it remains in the blood. The rats treated with gentamicin and triphala have shown significant increase in the urinary creatinine level while compared to gentamicin induced nephrotoxic rats. This protective effect of triphala may be due the prevention of the damage of the glomerular filtration apparatus and the normal filtration of creatinine across the glomerulus.

A significant increase in urinary glucose in gentamicin induced nephrotoxic rats is seen when compared to normal rats. This is due to the damage of Na^+ / Glucose symporter expressed on the apical surface of PCT cells by the aminoglycosides. This decreases the re- absorption of glucose by the Na^+ / Glucose symporter. Hence, there is increase in urinary glucose in the gentamicin induced nephrotoxic rats. There is significant decrease in the urinary glucose level in rats treated with triphala and gentamicin when compared to nephrotoxic rats. This may be due to the protective effect of triphala on Na^+ / Glucose symporter. The protected symporter allows the reabsorption of glucose along with sodium ions. Further, the rats treated with gentamicin have shown significant increase in the serum urea concentration when compared to normal rats. This is due to structural damage of the glomerular filter and leads to the accumulation of urea in serum. Hence, the serum urea concentration is more in gentamicin induced nephrotoxic rats.

The rats treated with triphala and gentamicins have shown significantly decreased serum urea concentration when compared to nephro toxic rats.¹¹

Also, increased malondialdehyde levels have been seen. Increased lipid peroxidation appears to be the initial stage to the tissue making it more susceptible to oxidative damage. The apical and basolateral membrane damage of the tubular cell is due to elevated levels of lipid peroxidation. This expressed in terms of TBARS. The TBARS level in rats treated with triphala and gentamicin is significantly reduced when compared to gentamicin induced nephrotoxic rats. This may be due to lipid peroxide free radical scavenging^{12, 13} activity of the triphala. Hence this leads to the protection of apical and basolateral membranes of the tubular cells. As various symporters and anti porters are expressed on these membranes, the normal function of the nephron gets resumed. These protective effects on the various transporters by triphala suggesting its nephroprotective effect.¹⁴

Further, histopathological examination shows restored renal parenchyma in triphala treated rats which may further strengthen this fact.

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