Abstract

The objective of this study was to determine nephroprotective effect on gentamicin, cisplatin, cyclosporine induced nephrotoxicity and lithium induced NDI in animal models. The herbal medicines continue to be the mainstay of healthcare systems and source of livelihoods of many communities in India and other countries. This leads to the increased dependence on medicinal products harvested from natural population. This dependence has led to local extinction of some important medicinal plants that include *Ficus racemosa*. This study has been designed to explore nephroprotective activity of ethanol and aqueous stem bark extracts (EFR and AFR) of *Ficus racemosa* in gentamicin, cisplatin, cyclosporine induced nephrotoxicity and lithium induced nephrogenic diabetes insipidus in experimental animals.

In gentamicin model after adjustment to the local environment, the rats were taken and divided them randomly to six groups (6 rats/group). Nephrotoxicity was induced with the administration of gentamicin (80mg/kg i.p.) for 8 days to the second group. The EFR and AFR (200 and 400mg/kg, b.w.) were administered to group III, IV, V, and VI respectively by oral route for three days and continued for another 8 days along with gentamicin. The protective effect was estimated by measuring the kidney weight, urine glucose, urine Na⁺, urine K⁺, urine volume, urine creatinine, serum urea, serum creatinine, lipid peroxidation, SOD, catalase, GSH and histopathological examination of kidney tissues in rats.

There was an insignificant difference in the weight of the isolated kidneys of the gentamicin induced nephrotoxic rats as compared to the normal rat. The gentamicin, EFR/AFR administered rats also showed an insignificant difference in the weights of the isolated kidneys when compared with gentamicin induced nephrotoxic rats. There was a significant increase in the urine glucose in nephrotoxic rats. The gentamicin, EFR/AFR administered rats also have shown a significant decrease in the urine glucose as compared to gentamicin induced nephrotoxic rats. An insignificant difference in the urine sodium was observed in gentamicin induced nephrotoxic rats. The gentamicin, EFR/AFR administered rats also showed an insignificant difference in the urine sodium when compared with nephrotoxic rats. An insignificant difference in the urine potassium in the gentamicin induced nephrotoxic rats...
was also observed when compared with the normal rats. The gentamicin, EFR/AFR administered rats also showed an insignificant difference in the urine potassium level. No change in urinary sodium and potassium levels was observed in the rats treated with gentamicin /EFR/AFR. The nephrotoxic rats have shown a significant decrease in the urine volume as compared to the normal rats. The gentamicin, EFR/AFR administered rats have shown an insignificant increase in the urine volume when compared with gentamicin induced nephrotoxic rats.

A significant increase in the urine urea was observed in the gentamicin induced nephrotoxic rats as compared to the normal rats. The gentamicin EFR/AFR administered rats have shown a significant decrease in the urine urea than the nephrotoxic rats. A significant increase in the urine creatinine was observed in the gentamicin induced nephrotoxic rats. The gentamicin, EFR/AFR administered rats have shown a significant decrease in the urine creatinine level. A significant increase in the urine and serum creatinine was also observed in the gentamicin induced nephrotoxic rats.

A significant increase in the serum urea was observed in the gentamicin induced nephrotoxic rats as compared to the normal rats. The gentamicin, EFR/AFR administered rats have shown a significant decrease in the serum urea than the nephrotoxic rats. A significant increase in the serum creatinine was observed in the gentamicin induced nephrotoxic rats. The gentamicin, EFR/AFR administered rats have shown a significant decrease in the serum creatinine than the nephrotoxic rats.

A significant increase in the lipid peroxide in the gentamicin induced nephrotoxic rats was observed as compared to the normal rats. The gentamicin/EFR administered rats have shown an insignificant decrease and gentamicin/AFR administered rats have shown a significant decrease in the lipid peroxide when compared to gentamicin induced nephrotoxic rats. A significant increase in the lipid peroxide was observed in the gentamicin induced nephrotoxic rats when compared with the normal rats. Significant decrease in the superoxide dismutase was observed in the gentamicin induced nephrotoxic rats. The gentamicin, EFR/AFR administered rats have shown significant increase in the SOD levels than nephrotoxic rats. A significant decrease in the superoxide dismutase in the gentamicin induced nephrotoxic rats was observed as compared to the normal rats. A significant decrease in the catalase was also observed in the gentamicin induced nephrotoxic rats as compared to the normal rats.
The gentamicin, EFR/AFR administered rats have shown significant increase in the catalase levels. A significant decrease in the catalase was also observed in the gentamicin induced nephrotoxic rats. A significant decrease in the catalase was noticed in the gentamicin induced nephrotoxic rats when compared to the normal rats. The gentamicin, EFR/AFR administered rats have shown a significant increase in the glutathione levels than nephrotoxic rats.

The histopathological changes were observed in the kidneys of gentamicin induced nephrotoxic rats as compared to the kidneys of normal rats with respect to parameters like glomerular congestion, glomerular hemorrhage, epithelial proliferation, tubular atrophy, tubular dilatation, denudation / necrosis, cast in tubules, hemosiderin in tubules, epithelial regeneration, interstitial inflammation and changes in the blood vessels. The rats of EFR/AFR administered along with gentamicin have shown protection against tubular dilatation, denudation/necrosis, and casts in the tubules.

In cisplatin model of study the mice were randomized and divided into 6 groups, each group consisting of 6 mice. Nephrotoxicity in mice was induced with the administration of cisplatin (12mg/kg). The stem bark ethanol and aqueous extracts of *Ficus racemosa* (EFR and AFR) were administered (200, 400mg/kg) to mice orally 1 h before the administration of cisplatin and at 24h and 48h after cisplatin injection. The parameters were studied 72 h after cisplatin administration. The protective effect was measured by estimating serum urea, serum creatinine, LPO, GSH and catalase.

A significant increase in the serum urea, creatinine, LPO and a significant decrease in the content of GSH and catalase were observed in cisplatin induced nephrotoxic mice. The cisplatin, EFR/AFR administered mice have shown noticeable alterations in these values suggesting the nephro protection.

In the cyclosporine model, the rats were divided by randomization into six groups of 6 animals each. The nephrotoxicity was induced to the group II rats by the administration of cyclosporine (50mg/kg p.o.) for 21 days. Cyclosporine (50mg/kg. p.o.) for 21 days + EFR/AFR (200 and 400mg/kg ) for 24 days was administered to the group III, IV, V and VI. Vehicle was administered to the group I. Twenty four hours after the administration of last dose; the sample blood was collected by cardiac puncture. This was utilized for the
estimation of the creatinine and BUN (blood urea nitrogen). The rats were sacrificed by euthanasia and the kidneys were isolated immediately. These were used for the determination of antioxidant properties. The biochemical parameters evaluated in this model are lipid peroxidation, superoxide dismutase, glutathione, and creatinine and catalase enzymes.

The increased serum creatinine was observed in the cyclosporine induced nephrotoxic rats. The cyclosporine, EFR/AFR administered rats have shown significant decrease in the serum creatinine as compared to the nephrotoxic rats. The serum urea level also increased significantly in the nephrotoxic rats when compared to the normal rats. The reduction in the levels of serum urea was observed in rats administered with the cyclosporine, EFR/AFR. The increased level in the lipid peroxide was observed in the nephrotoxic rats administered with cyclosporine than normal rats. The rats administered with cyclosporine, EFR/AFR have shown a significant decrease in the lipid peroxide when compared to cyclosporine induced nephrotoxic rats suggesting the nephroprotection.

A significant decrease in the superoxide dismutase was observed in nephrotoxic rats than the normal rats. An increased level in the SOD was observed in cyclosporine, EFR/AFR administered rats as compared to cyclosporine induced nephrotoxic rats. Decreased glutathione level was also observed in the cyclosporine induced nephrotoxic rats when compared to the normal rats. The cyclosporine, EFR/AFR administered rats have shown a significant increase in the glutathione levels when compared with cyclosporine induced nephrotoxic rats.

The noticeable decrease in the catalase was observed in the cyclosporine induced nephrotoxic rats. The cyclosporine, EFR/AFR administered rats have shown a significant increase in the catalase levels when compared cyclosporine induced nephrotoxic rats. A significant increase in the lipid peroxide level was observed in the nephrotoxic rats as compared to the normal rats. In the cyclosporine, EFR/AFR administered rats, a significant decrease in lipid peroxide was observed when compared to group II rats. A significant decrease in the superoxide dismutase, glutathione and catalase in the nephrotoxic rats was observed as compared to the decrease in normal rats. The rats administered with cyclosporine, EFR/AFR administered rats have shown a significant increase in these levels when compared to the nephrotoxic rats. A significant increase in serum creatinine level was observed in nephrotoxic rats (caused by cyclosporine) when compared to the normal rats. The
cyclosporine, EFR/AFR administered rats have shown a significant decrease in the serum creatinine.

The serum urea level was significantly increased in the cyclosporine induced nephrotoxic rats. The cyclosporine, EFR/AFR administered rats have shown a significant decrease in the serum urea when compared to cyclosporine induced nephrotoxic rats. The cyclosporine induced nephrotoxic rats have shown significantly decreased levels of serum urea when compared to the normal rats. The significantly decreased levels of lipid peroxide was showed by the rats administered with the cyclosporine, EFR/AFR administered rats when compared to the cyclosporine induced nephrotoxic rats.

The significantly decreased superoxide dismutase level was observed in the cyclosporine induced nephrotoxic rats when compared to the normal rats. The significantly increased superoxide dismutase level was observed in the cyclosporine, EFR/AFR administered rats when compared to the cyclosporine induced nephrotoxic rats. Significantly decreased levels glutathione was observed in the cyclosporine induced nephrotoxic rats when compared to the normal control rats. Significantly increased level of glutathione was observed in the cyclosporine, EFR/AFR administered rats when compared to the cyclosporine induced nephrotoxic rats. A significant decreased in the catalase levels in the cyclosporine induced nephrotoxic rats was observed when compared to the normal rats. A significantly increased level of catalase was observed in the cyclosporine, EFR/AFR administered when compared to the cyclosporine induced nephrotoxic rats (10.12 ± 0.2684 U/mg of protein). The results of this study reveal that the significant alterations in these biomarkers were observed in drug induced nephrotoxic experimental animal models when compared to the normal animals.

The nephrotoxic inducing drugs and EFR /AFR administered experimental animals have shown protective effects by altering these biomarkers towards the normal values indicating their nephron-protection. The result of histopathological examination also confirms the nephron-protection against drug induced nephrotoxicity. The nephron-protection effect of EFR/AFR may be due to the blockage of gentamicin, cisplatin and cyclosporine in entering the tubular cells. Hence these drugs in presence of EFR/AFR may get excreted out without producing damaging effects on the tubular cells of the kidney. This may also be due to the antioxidant constituents of the Ficus racemosa bark. The nephron-protective role may be due to the blockage of the cation macromolecule called megalin, which is expressed on the brush
border of the tubular cells of the nephron. This prevents pinocytosis of gentamicin, thereby prevents the formation of typical myeloid bodies within the tubular cells. The gentamicin molecule is also act on the calcium sensing receptors which are expressed on the apical membrane of the tubular cells. The activated receptors bring about cell signalling and cell death.

The EFR/AFR may act by inhibiting the calcium sensing receptor on the apical membrane. This mechanism may be responsible for their nephro-protection effect against gentamicin induced nephrotoxicity. This may be the possible role of EFR/AFR in protecting the gentamicin induced nephrotoxicity. It is reported that the CsA is able to generate oxygen species and lipid peroxidation, which are directly responsible for its nephrotoxicity. So, the use of antioxidants seems to be useful constituents’ helps to reduce the adverse effects like nephro-toxicity. The protective role of different antioxidants has been evaluated on CsA-induced kidney toxicity. It is the known factor that the antioxidants are the substances which are essential for the protection of organs like kidney. The plant shown kidney protections affect by virtue of its increased the antioxidant activity.

The EFR/AFR induced nephro-protection against lithium may be due to inhibitory effect on ENaC on the distal nephrons. This increases the inositol level and stimulates the cell cycle, thereby protects the nephrons. The transporters like, OCTs are expressed on the basolateral membranes of the tubular cells. These transporters are also responsible for the uptake of cisplatin into the tubular cells. Hence the injury due to cisplatin is due to the transporters like OCTs expressed on the basolateral membrane of the tubular cells. The cimetidine drug has antagonizing effect on OCTs. This OCTs inhibiting efficacy of cimetidine, could partially prevent the nephrotoxicity due to the administration of cisplatin. The uptake of this increases due to the over expression of OCT2 in the tubular cells, leading to the increased nephrotoxicity by cisplatin.

One of the possible mechanisms for the nephro-protection effect of Ficus racemosa bark extracts is due to the inhibitory effect on these transporters by the cispatin. This prevents the reuptake of cisplatin into the tubular cells. The protective effect against lithium induced nephrogenic diabetes insipidus may be due to the inhibitory effect of these extracts on ENAC
expressed on the tubular cells of DCT. This effect prevents the accumulation of lithium in the
DCT cells of the nephron. Thus this bark extracts helps to prevent this disorder. In the future,
the Ficus racemosa could constitute a novel herbal plant that will be useful for the treatment
of drug-induced nephrotoxicity and lithium induced nephrogenic diabetes insipidus.

**Key words:** Ficus ramosa, nephrogenic diabetes insipidus, lithium, cisplatin, gentamicin.