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RESEARCH ARTICLE
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Evaluation of nephroprotective potential of *Ficus religiosa* leaves against gentamicin induced nephrotoxicity in experimental albino wistar rats

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ABSTRACT

Objective: Ficus religiosa (Peepal) belonging to family Moraceae is one of the most versatile medicinal plants having a wide spectrum of biological activity and traditionally used to treat gonorrhoea, diarrhoea, and dysentery and other urogenital disorders, and kidney disorders. Ficus religiosa contained tannins, phenols, saponins, alkaloids, methionine, terpenoids, flavonoids, glycosides, proteins, essential and volatile oils and steroids. Previous pharmacological studies revealed that Ficus religiosa possessed antimicrobial, anti-amnesic, anticholinergic, antidiabetic, anti-inflammatory, analgesic, cytotoxic, anti-ulcer, wound healing, antioxidant, anti- asthmatic, hepatoprotective effects. The present study was carried out to evaluate the Nephroprotective potential of Ficus religiosa leaves on gentamicin induced nephrotoxicity in Albino Wistar rats.

Material and Methods: The plant material were collected, dried and authenticated from the CSIR-NSCAIR, New Delhi. HPLC studies were also performed. Albino Wistar rats weighing 150-200g of either gender were used in the study, after the approval of IAEC. The albino wistar rats were randomly divided into seven groups of 5 animals in each. Group I was administered normal saline for 14 days. Group I was administered normal saline for 14 days. Gentamicin (GM, 80mg/kg, i.p.) was administered from 8 day and induced till 14 days in Group II to Group VII. 200mg/kg (Group IV and Group VI) and 400mg/kg (Group V and Group VII) ethanolic extract of Ficus mature and young leaves and 500mg/kg (Group III) of cystone as a standard was treated to rats 1 hr. before GM administration. After body weight measurements. The urine of each group of rats was collected and the volume was measured over 24 hrs. On 15 days blood samples were collected and sacrificed. Sera were collected for biochemical analysis (serum urea, Serum creatinine, uric acid, albumin, albumin, total proteins level and Sodium, Potassium, Chlorides). The animals were sacrificed after anesthesia. The left kidney was excused for tissues homogenates that were used for biochemical parameters (Malondialdehyde, Superoxide dismutase, Glutathione, Catalase) and right kidney were used for

histopathological studies.
Result: The phytochemical and HPLC analysis were measured. Treatment with ethanolic extract of FML & FYL (200mg/kg,400mg/kg) significantly improved the altered levels of Serum Creatinine, Serum Urea, Uric acid, Albumin, Total proteins, Sodium, Potassium and Chlorides as compared to disease control groups. It also significantly restored the altered levels of MDA, SOD, GSH and CAT in renal tissues. Apart from these, extract of Ficus religiosa leaves also diminished histopathological alterations induced by GM in kidney injury.
Conclusion: The result of this study observed that the ethanolic extract of FML and FYL has a significant nephroprotective effects against gentamicin –induced nephrotoxicity in albino wistar rats.
Keywords: *Ficus religiosa,* IAEC, Nephroprotective, GM, phytochemical, biochemical parameters, histopathology, antioxidant activity, HPLC.

INTRODUCTION

Drug-induced nephrotoxicity is defined by the presence of any kidney injury caused directly or indirectly by medication. The presentation varies from an acute or chronic reduced glomerular filtration rate (GFR) to nephrotic syndrome related, respectively, to glomerular and tubular damage. Drug-induced acute renal failure (ARF) accounted for 20% of all ARF cases in an Indian study. Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66%, due to a higher incidence of diabetes and cardiovascular diseases compelling them to take multiple medications [1]. Acute Kidney Injury (AKI) is defined by oliguria accompanied by a rise in serum creatinine (sCr) within 48 h of either 26.5 mol/L (0.3 mg/dL) or 50% as compared to baseline values during the first week of exposure to a potentially nephrotoxic agent [12,13]. Antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), anti-neoplastic and some immunomodulatory, and antihypertensives drugs are among the most frequently encountered nephrotoxic drugs [2-4]. AKI is a global health challenge of vast proportions, as approximately 13.3 million people worldwide are affected annually. AKI has a high mortality rate with 1.7 million deaths per year. [5-7]. In spite of inducing nephrotoxicity and Ototoxicity, gentamicin is used clinically due to its wide spectrum of activities against gram-negative bacterial infections caused by Pseudomonas, Proteus, and Serratia [8-10]. Gentamicin induces nephrotoxicity via nitrosative stress. It also causes nephrotoxicity by inhibiting protein synthesis in renal cells. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure [11].

Medicinal plants are rich source of phenolic compounds and have large number of biological effects including antioxidant activity which may help to protect the cells against the oxidative damage caused by free radicals (Berry 1996) [12]. Ficus religiosa Linn. belong to family Moraceae are medicinally important plants, wildly grown in some parts of India and used in the treatment of various disease and disorders of human ailments by tribal and rural people of our country [13]. All parts (Bark. Leaves, root, fruit) of F. religiosa contains many major bioactive compounds such as lanosterol, β –sitosterol, bergaptol, bergapten, steroids, flavonoids, alkaloids, phenolic compounds, tannins, flavonoids (kaempeferol, quercetin) etc due to the presence of these major bioactive chemical constituents the tree possess various major pharmacological activity such as anti-diabetic activity, anti-convulsant, anti-oxidant, anti-inflammatory, wound healing, anti-microbial, anti-cholinergic activity, and also useful in the treatment of various diseases and disorders includes ulcers, skin diseases, epilepsy, Gastro Intestinal (GI) disorders, infectious, amnesia, asthma [14- 16].

METHODOLOGY:

1. MATERIAL

1.1. Drugs and Chemicals

Ethanol (Chongshu Hongshong Fine Chemical Co. Ltd, Chongshu city, New Delhi), phosphate buffer, Gentamicin injection I.P.(Genticyn 80mg/2ml, Abott), formalin solution, paraffin, H and E stain, Ketamine, formaldehyde (RFCL Limited, Gujarat), TBA (CDH, New Delhi), GAA (Thermofisher Scientific India Pvt. Ltd. Mumbai),GSH (CDH, New Delhi), DTNB (CDH, New Delhi), Hydrogen peroxide (Thomas Baker, Mumbai), sodium carbonate (RFCL Limited, Gujarat),

ethylenediamine tetra acetic acid , Nitrobluetetrazolium (CDH, New Delhi), Triton-X-100(Sisco Research Laboratories Pvt. Ltd. Maharashtra), Hydroxylamine hydrochloride(RFCL Limited, Maharashtra). All drugs and solvents were of analytical grade.

1.2. Collection and authentication of plant:

The *Ficus religiosa* leaves were collected from Lucknow, India. The collected plant materials were taxonomically identified and authentication has been done by botanical Dr. Sunita Garg Chief Scientist Head, RHMD, CSIR, NISCAIR, and New Delhi. The specimen was submitted for future reference NISCAIR/RHMD/Consult/2021/3819-20, at Raw Materials Herbarium and Museum, Delhi (RHMD).

1.3. Preparation of Ethanolic extract of Ficus religiosa leaves:

The leaves of *Ficus religiosa* were cleaned and removed foreign particles. It was dried at room temperature and made into coarse powder by using grinder. About 500gm of grinded material was liable to extraction with ethanol (70%) as a solvent by using Soxhlet apparatus at room temperature to obtain a viscous mass**.** Then, samples were filtered using filter paper and concentrated using vacuum rotary evaporator after dried on a dissipating dish at temperature upto 30^0C to - 40^0C to a semisolid strong frame. A sticky semi-strong greenish substance was acquired.

1.4 Phytochemical Screening:

Preliminary Phytochemical screening for alkaloids, glycosides, tannin, saponins, flavonoids steroids, terpenoids, protein and amino acid and carbohydrates.

1.5 HPLC Analysis:

The extract composition analysis was performed using a rapid chromatographic method based on the use of a column with solid fused-core particles (Xbridge C18, 5 μm, 2500 mm x4.6 mm, Waters corporation, USA) operating at a relatively room temperature. Ultra-pure water (solvent A) and acetonitrile (solvent) at 1.0 mL per min. were used as the mobile phase. The separation gradient was as follows: 0 min: 95% A, 10 min: 70%A, 14 min: 40% A, 16min: 40%A, 24 min:20% A, 32min: 20% A, 35 min: 95% A, 40 min: 95% A. This gradient includes column cleaning (2.5 min.) and column equilibration time (2min.). The PDA detector collected the data between 210 and 400nm, and the chromatograms were processed at 270 nm and 325 nm. The injections (5 mL) were programmed to occur at half the time of each extract fraction collection to represent the average fraction composition (every 10 min.). This HPLC procedure separation that combined with the 1^{st} dimension separation composes the bidimensional chromatogram that characterizes the sample.

2. Pharmacological screening;

2.1 Experimental Animals:

Albino wistar rats weighing (150-200gm) of either gender were used in the study, after the approval of IAEC (IAEC No.) All animals were initially acclimatized to the laboratory environment for 10 days prior to their use. The animals were housed in polypropylene cage. They were housed in a clean cage. The bedding materials of the cages were changed at alternate day. The standard laboratory conditions were maintained at 12:12 hours light and dark cycles, at room temperature 25 ± 2^0C and a relative humidity 40-50%. The rats were fed with standard pellet diet and water ad libitum.

2.2 Experimental design and treatment of animals:

The albino wistar rats were divided into seven groups (five animals in each group) used for the experimental study. The treatments were administered for the duration of 14 days.

Group I (NC): The rats were administered orally with normal saline for 14 days.

Group II (DC): The rats treated with gentamicin (80mg/kg, i.p.) for first eight days.

Group III (STD): The rats were administered with gentamicin (80mg/kg. i.p.) for eight days and Cystone (500mg/kg.) by oral route for 14 days.
Group IV (T1): The rats were administered with gentamicin (80mg/kg, i.p.) for eight days and EEFML (200mg/kg) by oral route for 14 days.
Group V (T2): The rats were administered with gentamicin (80mg/kg, i.p.) for eight days and EEFML (400mg/kg) by oral route for 14 days.
Group VI (T3): The rats were administered with gentamicin (80mg/kg, i.p.) for eight days and EEFYL (200mg/kg) by oral route for 14 days.
Group VII (T4): The rats were administered with gentamicin (80mg/kg, i.p.) for eight days and EEFYL (400mg/kg) by oral route for 14 days.

2.3 Induction of Nephrotoxicity by Gentamicin
Gentamicin was used for induction of nephrotoxicity. Gentamicin was used in 80mg/kg dose and it was administered by i.p. route (intraperitoneal route). The dosing was repeated every day for a duration of eight days. The process of dose administration was performed in the morning every day at the same time.

2.4 Sample Collection
One day before the euthanasia, the animals were housed in metabolic cages. The urine of each rat was collected and the volume was measured over a 24 hrs. Period. On the 14^{th}day, all rats were anaesthetized with Ketamine (75mg/kg, i.p.) and euthanized. The cardiac blood samples were collected into non-heparinized tubes. The collected blood samples were kept for 30 minutes at 25^0C for clotting, followed by centrifugation at 2000rpm for 15 minutes. Sera were collected and stored frozen for biochemical analysis. The animals were sacrificed after anesthesia and the then the lower abdomen was opened. After the gentle removal of perirenal fat, the kidneys were weighed using a digital balance. The left kidneys were excused immediately, washed in normal saline (0.9%w/v), homogenized in 0.1M phosphate buffer (Ph-7.4) to get tissue homogenates that were then used for biochemical parameters. The right kidney was used for histopathological studies.

2.5 Physical Analysis:
2.5.1 Body Weight: The body weight of albino wistar rats was evaluated before starting and at the end of the study and % changes in body weight were calculated.
2.5.2 Kidney Weight: The kidney weight of the albino wistar rats was measured at the end of the study.
2.5.3 Urine Volume: The urine volume of albino wistar rats was measured.
2.6 Biochemical Parameters on serum blood:
2.6.1 Serum creatinine: Serum creatinine level was evaluated by Mod. Jaffe's Kinetic method using creatinine kit.
2.6.2 Serum urea: Serum urea level was evaluated by GLDH Kinetic method using kit.
2.6.3 Serum uric acid: Serum uric acid level was evaluated by Uricase /PAP method using kit.
2.6.4 Serum albumin: Serum albumin was evaluated by Bromocresol Green method using kit.
2.6.5 Total Protein: Serum albumin was evaluated by Biuret method using kit.
2.6.6 Serum BUN: Serum BUN was evaluated by Abbexa's BUN Assay Kit.

2.7 Antioxidant Parameters on renal tissue:
A hematoxyline and eosin stain was used for histopathological studies. Fixed tissues sample with 10% formaldehyde were dehydrated in a series of ascending graded ethanol, and finally embedded in paraffin wax. After that, paraffin sections of kidney were sectioned at 5µm thickness using a microtome and stained with hematoxyline and eosin stain.

2.7.1 Assessment of MDA: The concentration of MDA on renal tissue of Albino Wistar Rats was determined by Thiobarbituric acid (TBA) assay. Take 1mL of supernatant in 1mL of 0.67%(w/v) of TBA was added and boiled on a water bath f0r 10 minutes, cooled and diluted with distilled water. Blank were prepared by adding all the reagents except the supernatant of renal tissue. The absorbance was measured

by spectrophotometrically at 535 nm.

2.7.2 Assessment of CAT: For the assessment of concentration of MDA on renal tissue of Albino Wistar Rats, 1000µL of tissue homogenate was subjected with 3900µL of 50mM phosphate buffer of pH neutral and 2000µL of Hydrogen peroxide (H_2O_2) 30mM (1.75 mL H_2O_2 in 20mL Distilled Water). The absorbance was measured thrice at an interval of 15 sec. at 240nm.

2.7.3 Assessment of SOD: Take 650µl of PBS in a set of test tubes**.** Add 30µL MTT [3-(4, 5-dimethyl thiazol-2-4)2, 5-diphenyl tetrazolium bromide] and then, in both the test tubes 75µL of pyrogallol was added. After the mixture was incubated for 5 minutes at room temperature. To restrict this reaction, 750µL of dimethyl sulfoxide was added to both the tubes and 10µL of sample was added to second tube. The absorbance was measured at 570nm.

2.7.4 Assessment of GSH: Take 0.2mL of supernatant of renal tissue of rats, 2.5mL of Tris-buffer and 0.2mL of DTNB [5, 5 –dithibis (2-nitrobenzoic acid)] reagent was added and mixed. Absorbance was measured at 412nm.

2.8 Histopathological studies:
A hematoxyline and eosin stain was used for histopathological studies. Fixed tissues sample with 10% formaldehyde were dehydrated in a series of ascending graded ethanol, and finally embedded in paraffin wax. After that, paraffin sections of kidney were sectioned at 5µm thickness using a microtome and stained with hematoxyline and eosin stain.

Statistical analysis:
All data were expressed as mean ±SEM (Standard Error of the mean). The statistical result were evaluated by using one-way ANOVA that is followed by Tukey multiple compare tests $P<0.05$ were considered as statistically significant.

2.9 RESULT:
2.9.1 Preliminary Qualitative test for EEFML and EEFYL

Table 1.1 phytochemical Analysis of Extract:

S.NO.	Phytochemical Constituents	Experiment	Inference EEFML	Inference EEFYL
1.	**Alkaloids**	Mayer's test	++	+
		Dragendroff's test	++	+
2.	**Glycosides (Anthraquinone glycosides)**	Borntrager's test	++	+
3.	**Tannins**	Gelatin test	++	+
4.	**Saponins**	Foam test	++	+
		Frothing test	++	+
5.	**Flavanoids**	Lead acetate test	++	+
6.	**Steroids**	Libbermann buchard's test	++	+
7.	**Terpenoids**	Chloroform test	++	+
8.	**Protein and Amino acids**	Ninhydrin test	++	+
		Biuret test	++	+
9.	**Carbohydrates**	Molisch's test	++	+
		Fehling's test	++	+

EEFML; Ethanolic Extract of *Ficus* Mature Leaves, EEFYL; Ethanolic Extract of *Ficus* Young Leaves, ++Prominent, + moderate.

2.9.2 HPLC analysis:

An HPLC analysis was performed to estimate the EEFYL. The chromatogram of EEFYL depicts R_T 2.490, 18.04 and 22.872 with % area 8.64, 7.65 and 83.70 respectively.

Table no.1.2 Retention time, Peak area, height & % area of EEFYL

SI.No.	RT	Area	Height	% Area
1	2.490	1314026	260598	8.64
2	18.040	1163614	140265	7.65
3	22.872	12724802	1449206	83.70

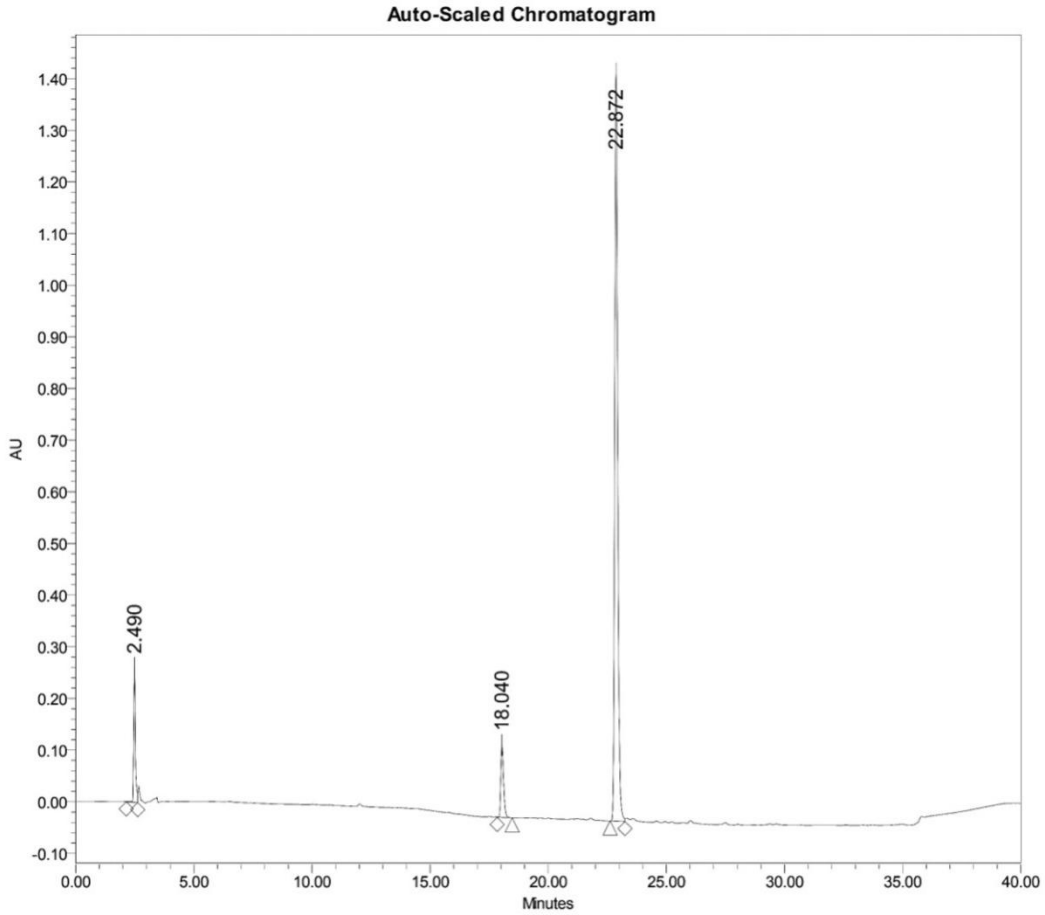

Fig. 1.1 Chromatogram showing different Peaks of EEFYL

Table 1.3 Retention time, Peak area, height & % area of EEFML

SI.No.	RT	Area	Height	%Area
1	2.491	1319971	238529	8.47
2	18.039	1006775	123999	6.46
3	22.866	12242983	1405381	78.55
4	35.799	1015553	12499	6.52

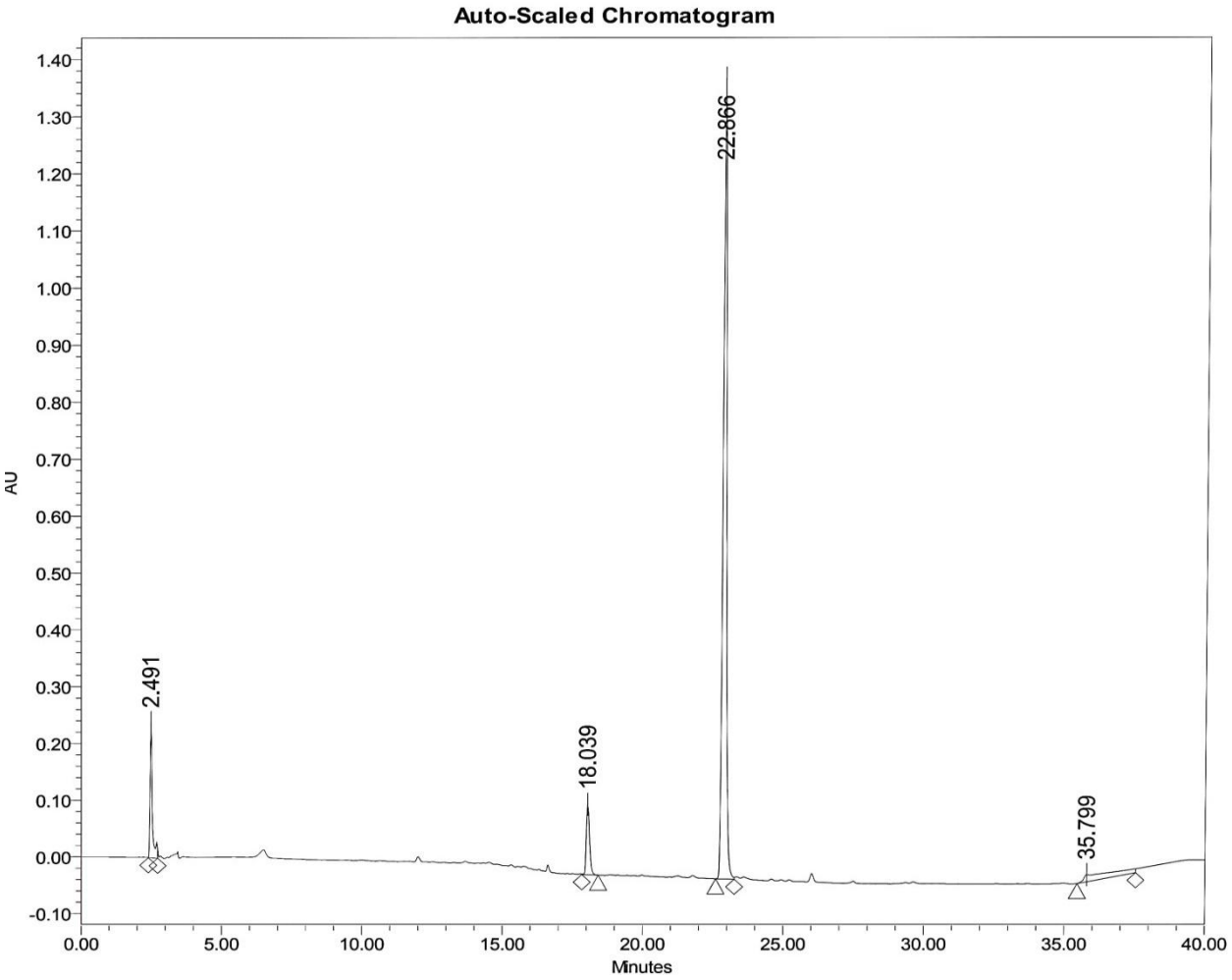

Fig.1.2 Chromatogram showing different Peaks of EEFML

2.9.3 Effect of EEFML & EEFYL on blood serum parameters against gentamicin induced nephrotoxicity in rats: Evaluation of serum parameters to illustrate nephroprotection properties of Ethanolic extract of Ficus mature leaves and Ficus young leaves against gentamicin induced nephrotoxicity.

The effect of EEFML & EEFYL treatments on serum creatinine, uric acid, Urea, BUN, Albumin and Total Protein are shown in Table no. 1.4. The Gentamicin treatment significantly increased the concentration of serum creatinine, uric acid, Urea, BUN, Albumin and Total Protein in disease control animal when compared to normal control animals. The EEFML & EEFYL revert the level of serum creatinine, uric acid, Urea, BUN, Albumin and Total Protein in a dose dependent manner. EEFYL at a dose 400mg/kg was reduced the level of serum creatinine, uric acid, Urea, BUN, Albumin and Total Protein ($P<0.001$, $P<0.01$, $P<0.05$) significantly, when compared to disease control group animals. The standard drug cystone also revert the level of serum creatinine, uric acid, Urea, BUN, Albumin and Total Protein ($P<0.001$, $P<0.01$) significantly.

Table 1.4 Effect of EEFML and EEFYL on blood serum parameter against gentamicin induced nephrotoxicity in rats

Groups	Treatment	Creatinine (mg/dL)	Uric acid (mg/dL)	Urea (mg/dL)	BUN (mmol/L)	Albumin (mg/dL)	Total Protein (mmol/L)
Normal Control	Normal Saline	0.57±0.02	1.59±0.04	35.84±0.66	3.59±0.03	4.08±0.17	7.36±0.06
Disease	Gentamicin	0.97±0.03	2.60±0.02	75.10±0.33	8.44±0.06	9.14±0.42	8.53±0.04

Control	(80mg/kg)						
Standard	Cystone (500mg/kg) + GM (80mg/kg)	0.68±0.01***	1.35±0.02***	55.65±0.09***	5.36±0.04***	7.22±0.28**	8.22±0.06**
Test 1	EEMYL (200mg/kg) + GM(80mg/kg)	0.93±0.01	2.55±0.09	71.27±1.01	8.03±0.16	7.67±0.36	8.36±0.06
Test 2	EEMYL (400mg/kg) + GM(80mg/kg)	0.87±0.02*	2.36±0.03*	68.54±1.28*	7.56±0.22**	7.84±0.35**	8.28±0.05*
Test 3	EEFYL (200mg/kg) + GM(80mg/kg)	0.92±0.01	2.52±0.05	72.73±0.66	7.27±0.14	7.84±0.39	8.40±0.06
Test 4	EEFYL (400mg/kg) + GM(80mg/kg)	0.85±0.02**	2.30±0.03**	66.75±1.85**	7.15±0.16*	7.38±0.30*	8.20±0.05**

2.9.4 Effect of EEFML & EEFYL on Sodium, Potassium and Chloride level in urine Evaluation of gentamicin induced nephrotoxicity in rats.

The effects of EEFML & EEFYL treatment on urinary concentration of sodium, potassium and chloride are shown in Table no.1.5. Gentamicin administration significantly increased the concentration of sodium and decreased the concentration of potassium and chloride in rats as compared to normal control group. The treatment of EEFML & EEFYL at a dose 400mg/kg significantly ($P<0.01$, $P<0.05$) decreased the rise in concentration of sodium and increased the decline in the concentration of potassium and chloride when compared to disease control group animals. The standard drug cystone also significant ($P<0.001$) decreased the level of sodium and significant ($P<0.001$) increased the level of potassium and chloride.

Table 1.5 Effect of EEFML & EEFYL on sodium, potassium and chloride in urine against gentamicin induced nephrotoxicity in rats.

Groups	**Treatment**	**Urine**		
		Sodium	**Potassium**	**Chloride**
Normal Control	Normal Saline	141.74±1.39	6.19±0.20	108.45±0.66
Disease Control	Gentamicin(80mg/kg)	188.26±1.19	3.85±0.21	98.72±0.48
Standard	Cystone(500mg/kg)+ GM(80mg/kg)	168.04±1.07***	5.45±0.20***	105.04±0.65***
Test 1	EEFML(200mg/kg)+ GM(80mg/kg	186.76±1.48	4.04±0.17	100.03±0.74
Test 2	EEFML(400mg/kg)+ GM(80mg/kg)	180.02±1.13**	4.73±0.15*	102.98±0.69**
Test 3	EEFYL(200mg/kg)+ GM(80mg/kg	183.64±1.47	4.24±0.17	100.87±0.74
Test 4	EEFYL(400mg/kg)+ GM(80mg/kg)	181.75±1.12*	4.98±0.15**	102.82±0.69**

2.9.5 Tissue Antioxidant Analysis:

The effects of EEFML & EEFYL treatment on Malondialdehyde, catalase Superoxide dismutase and Glutathione level of the renal tissue are shown in Table no.1.6. The results showed that administration of gentamicin caused remarkable increase in MDA level of the renal tissue in comparison of control group. Pre-treatment of EEFML & EEFYL at a dose of 400mg/kg one hour before the administration of gentamicin has prevented the gentamicin-induced nephrotoxicity caused a significant ($P<0.05$) decline in the MDA level of the renal tissue when compared with disease control group. The standard drug cystone also decreased in the MDA level of the renal tissue. While the co-administration of gentamicin caused significant decline in Catalase level of the renal tissue compared with control group animals. Pre-treatment of EEFML & EEFYL rises (non-significantly) at a dose 400mg/kg one hour before the administration of gentamicin inhibited the gentamicin-induced nephrotoxicity. The standard drug cystone decreased MDA level and increased the CAT level of the renal tissue in rats significantly ($P<0.01$). From the results, it is clear that rats in disease control group exhibited a decrease in renal SOD and GSH levels

when compared to normal control group and indicating negative effects of gentamicin administration on renal tissues. Therefore, the positive impacts are assisted by fact that pretreated with EEFYL at a dose 400mg/kg significantly (P<0.05) improved the SOD. The standard drug cystone significant (P<0.01) rise the SOD and GSH level of the renal tissue in rats.

Table 1.6 Effect of EEFML & EEFYL of MDA, CAT, SOD and GSH level on renal tissue against gentamicin induced nephrotoxicity in rats.

Groups	Treatment	MDA (nmol/mL)	CAT (U/mg per Protein)	SOD (U mg Protein)	GSH (Ug/mg P)
Normal Control	Normal Saline	2.89±0.25	9.57±0.45	9.62±0.10	35.3±0.43
Disease Control	Gentamicin (80mg/kg)	5.68±0.37	2.04±0.32	5.36±0.05	24.3±0.35
Standard	Cystone(500mg/kg)+ GM (80mg/kg)	3.96±0.32**	4.04±0.36**	5.86±0.11**	26.04±0.24**
Test 1	EEFML(200mg/kg)+ GM (80mg/kg)	5.18±0.23	3.47±0.34	5.47±0.07	24.48±0.22
Test 2	EEFML(400mg/kg)+ GM (80mg/kg)	4.25±0.23*	3.76±0.36*	5.74±0.06*	24.98±0.23
Test 3	EEFYL(200mg/kg)+ GM (80mg/kg)	5.25±0.24	3.52±0.34	5.46±0.07	25.24±0.22
Test 4	EEFYL(400mg/kg)+ GM (80mg/kg)	4.37±0.22*	3.04±0.36	5.76±0.06*	25.77±0.23*

2.9.6 Histopathological studies

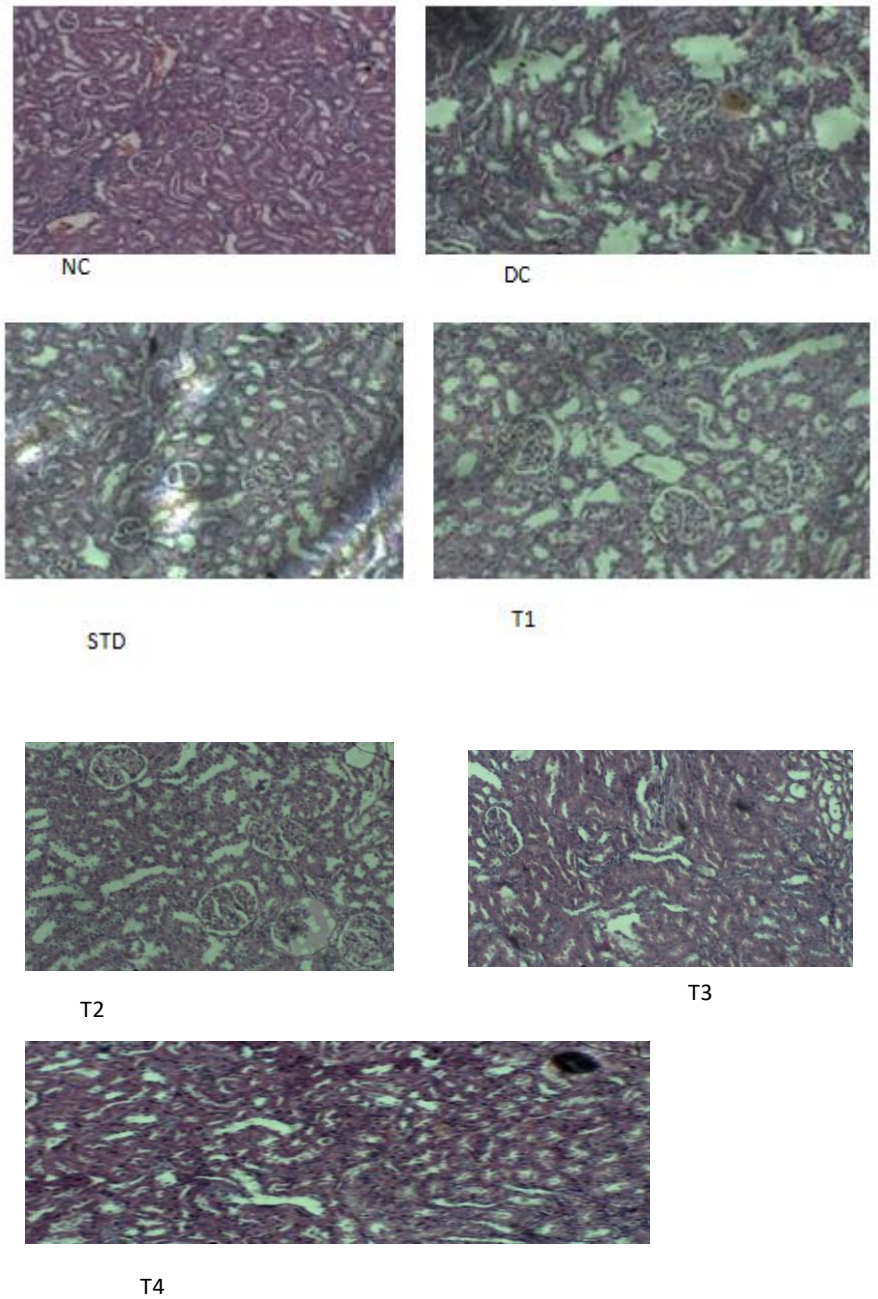

Fig.1.3 Effect of EEFML & EEFYL on histopathology of gentamicin induced nephrotoxicity in wistar rat's kidney (Hematoxyline & eosin stain, X40.)

It represents **Normal control group (NC)** showing Normal glomeruli and tubules when compare to disease control group.

Disease control group (DC) showing degenerative tubular structures, tubular dilatation, glomerular atrophy, necrosis and narrowing of the bowman's space.

Standard group (STD) showing reduced the renal tubular and some of them also show intracellular edema membrane, tubular dilatation and glomerular atrophy when compared to disease control kidney sections.

Treatment: Test 1 (T1) & **Test 3** (T3) EEFML & EEFYL (200mg/kg, p.o.) + GM (80mg/kg, i.p.), **Test 2** (T2) & **Test 4** (T4) EEFML & EEFYL (400mg/kg, p.o.) +GM (80mg/kg, i.p.) showing reduced the renal tubular and some of them show intracellular edema membrane, tubular dilatation and glomerular atrophy when compared to disease control kidney sections.

2.9.7 Body weight, Kidney weight and Urine Volume analysis:

The effects of **EEFML &** EEFYL on Body Weight, Kidney Weight and Urine Volume were shown in Table 1.7. A significantly decreased in the body weight was observed in gentamicin administered group as compared to normal control group. Table no. 1.7 showed the effect of EEFML & EEFYL on the body weight changes. The results were showed that Pre-treatment with EEFML & EEFYL at a dose 400mg/kg, a significant ($P<0.01$) elevated body weight in standard and test groups were observed when compared to disease control group. The results of this study showed that gentamicin administration significantly decline the level of urine volume thus induced nephrotoxicity in the disease control group when compared to normal control group, thus induced nephrotoxicity. The treatment with EEFML & EEFYL at a dose 400mg/kg result in significant ($P<0.01$, $P<0.05$) elevated the level of Kidney Weight and Urine Volume in comparison to disease control group animals. The standard drug cystone also significant ($P<0.001$) increased the level of urine volume when compared to disease control group.

Table 1.7 Effect of EEFML & EEFYL on Body weight changes (%), Kidney weight and Urine volume against gentamicin induced nephrotoxicity in rats

Groups	Treatment	Body weight Changes in (%)	Kidney Weight (g)	Urine Volume (mL)
Normal Control	Normal Saline	2.86±0.12	0.65±0.02	6.03±0.12
Disease Control	Gentamicin (80mg/kg)	-6.84±0.17	0.88±0.03	2.59±0.10
Standard	Cystone(500mg/kg)+ GM (80mg/kg)	-5.38±0.22***	0.74±0.02***	4.77±0.10***
Test 1	EEFML(200mg/kg)+ GM (80mg/kg)	-6.46±0.25	0.82±0.03	4.26±0.15
Test 2	EEFML(400mg/kg)+ GM (80mg/kg)	-5.54±0.23**	0.78±0.01*	4.61±0.15*
Test 3	EEFYL(200mg/kg)+ GM (80mg/kg)	-6.48±0.24	0.86±0.01	4.28±0.15
Test 4	EEFYL(400mg/kg)+ GM (80mg/kg)	-5.52±0.23**	0.76±0.01**	4.63±0.16**

3. Discussion:

Acute Kidney injury commonly referred as acute renal injury, the term used to describe as a rapid decline in the glomerular filtration rate. The purpose of study for evaluation of nephroprotective potential effect of *Ficus religiosa* leaves on gentamicin induced nephrotoxicity in albino wistar rats. Independent of cell injury, gentamicin blocks a variety of basolateral and brush border membrane transporters, causing irregularities in electrolytes (**Perazella *et al,* 2018 [6].** Transport inhibition impairs tubular reabsorption as well as cell viability, which leads to necrosis or apoptosis in the end. The increase the levels of GM in kidney tissue damages the tubular cells, which leads to hyponatremia, excessive water consumption, significantly reduced body weight gain, and urinary sodium volume.

MDA is a physiological agent that is created as a byproduct of arachidonic acid metabolism by the peroxidative breakdown of unsaturated lipids. The cause of GM-induced nephrotoxicity has been linked to ROS, notably hydroxyl radical (Son et al, 2013). In the renal parenchyma, GM treatment has been shown to increase the production of ROS and speed up the LPO of bio- membranes (**Baliga *et al*., 1999).**

Reduced glutathione is an important cellular antioxidant and is also a substrate for the glutathione peroxidase, which provide a mechanism for the detoxification of xenobiotic.

These decreases in renal antioxidant enzymatic protection could aggravate the oxidative damage. The increased production of ROS in GM-induced nephrotoxicity may cause inactivation of antioxidant enzymes such as GSH. SOD is an enzyme associated with copper, zinc, and manganese which are

essential for enzyme activity **(18).** It is said that SOD has the distinct ability to neutralize superoxide, one of the most damaging free radical substances in nature. The decreased SOD activity is not enough to scavenge the superoxide anion produced during the normal metabolic process and cause the initiation and propagation of lipid peroxidation in GM-treated. The activities of CAT and GSH-Px also decreased in the GM-treated group, which resulted in the decreased ability of renal to scavenge toxic hydrogen peroxide and lipid peroxidation. Therefore, the significant increase in the level of MDA, as marker of lipid peroxidation, was observed in the GM-treated groups (**Son *et al* 2013, [18**].

The histopathological analysis were observed that various necrosis, proximal convoluted show loss of border and loss of nuclei in some epithelial cells in disease control group, whereas, in animals treated with Ficus religiosa extract were improved of all alteration in renal injury. EEFML & EEFYL at a dose 400mg/kg was reduced the level of serum creatinine and uric acid ($P<0.01$, $P<0.05$) significantly, when compared to disease control group animals. EEFML & EEFYL at a dose 400mg/kg lowered ($P<0.01$, $P<0.05$) significantly the level of serum urea and BUN, when compared to disease control group animals. EEFML & EEFYL at a dose 400mg/kg significantly inhibited the increases in the serum concentration of albumin and total protein when compared to disease control group animals. The treatment of EEFML & EEYFL at a dose 400mg/kg significantly ($P<0.01$, $P<0.05$) decreased the rise in concentration of sodium and increased the decline in the concentration of potassium and chloride when compared to disease control group animals. Nephroprotective effects of EEFML & EEYFL shows improved ameliorative action on the kidneys. The observations of also supports our findings by reporting the ameliorative effect of EEYFL on electrolyte abnormalities in GM intoxicated rats. EEFML & EEFYL significantly prevented the loss in body weight induced by gentamicin. It could then be suggested that EEFML & EEYFL prevents proteolysis or even potentiate protein synthesis. The hypothesis of inhibition of proteolysis is supported by the fact that EEFML & EEFYL significantly prevented the drop in serum protein content induced by gentamicin. The results were showed that Pre-treatment with EEFYL at a dose 400mg/kg, a significant ($P<0.001$, $P<0.01$) elevated body weight in test groups were observed when compared to disease control group. Pre-administration of EEFML & EEFYL at a dose 400mg/kg declined in the rise in kidney weight significantly ($P<0.01$, $P<0.05$) when compared to disease control group. The treatment with EEFML & EEFYL at a dose 400mg/kg result in significant ($P<0.001$, $P<0.01$, $P<0.05$) elevated the level of urine volume in comparison to disease control group animals. The high MDA level observed in the group of rats treated only with GM significantly decrease ($P < 0.05$) in rats receiving EEFML & EEYFL at the same time. The lower MDA level in the GM+ EEFML & EEFYL group, which was close to the control value, indicates the EEFML & EEFYL attenuation of LPO. This inhibitory effect of EEFML & EEFYL might be the result of its ability to interrupt the propagation of the free radical chain reaction already initiated in the membrane lipids of kidney lysosomes and other sub-cellular organelles. In our present study EEFML & EEFYL significantly ($p<0.05$) elevated the level of SOD, Catalase, GSH and MDA concentrations. In histopathological studies, EEFML & EEFYL 200mg/kg, 400mg/kg treated with test groups the alterations in renal damage were considerably improved. The result of the present study states that EEFML & EEFYL has the ability to improved renal injury induced by GM. The Phytochemical Studies showed that the presence of various phytoconstituents such as flavonoids, steroids, alkaloids, Triterpenoids and Vitamin K which are assumed to be reliable for its nephroprotective activity.

4. Conclusion:

The present study was aimed to evaluate the nephroprotective potential effect of *Ficus religiosa* leaves on gentamicin induced nephrotoxicity in albino wistar rats. As gentamicin nephrotoxicity is a reversible process depending on the level of renal injury. It is accomplished that GM generates renal toxicity due to the formation of ROS. GM increases lipid peroxidation, GSH depletion, body weight and increases the level of creatinine and urea in serum as well as leads to the glomerular congestion by destruction of glomeruli and loss of Bowman's space. The results justify the traditional uses of Ficus religiosa prevention on renal disorders. The effect of ethanolic extract of *Ficus religiosa* due to the presence of flavonoids, stigmasterol, vitamin K that shown ameliorative effect on GM toxicity. The antioxidant effect of *Ficus religiosa* strengthened the preventive action on nephrotoxicity.

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