International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 595-614 www.ijprajournal.com ISSN: 2456-4494

Investigation of Nephroprotective activity of Justicia Gendarussa Leaves Extract against Gentamicin Induced Nephrotoxicity in Rats.

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Submitted: 05-11-2022 Accepted: 20-11-2022

ABSTRACT
In the current study, simultaneous treatment with ethanolic leaves extracts of Justicia gendurussa Burm F provided a marked functional and histological protection against acute renal damage in rats treated with gentamicin. Increase in blood urea nitrogen, serum urea and serum creatinine induced by gentamicin was prevented by ethanolic leaves extracts of Justicia gendurussa Burm F. This study revealed that orally-administered ethanolic leaves extracts of Justicia gendurussa Burm F had a significant and dose dependent protective effect in gentamicin-induced nephrotoxicity in rats. A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models. A significant increase in lipid peroxidation products malondialdehyde (MDA) in gentamicin injected rats, suggesting the involvement of oxidative stress. A role of lipid peroxidation in gentamicin-induced acute renal failure has also been described in previous studies. Moreover, pretreatment of rats with hydroxyl radical scavengers has been shown to protect against gentamicin induced acute renal failure. Gentamicin is known to decrease the activities of catalase, and the level of SOD. Garcinol is the main medicinal component of the dried fruit rind of Justicia gendurussa Burm F. In vitro studies of garcinol revealed its potential therapeutic effects, such as its anti-oxidative, anti- inflammatory and anti-cancer properties. Similarly, in vivo studies in animal models also demonstrated the efficacy of garcinol for the treatment of various inflammatory and cancerous conditions. Garcinol has been proposed to react with peroxyl radicals by a single electrontransfer followed by deprotonation from the hydroxyl group of the enolized 1, 3-diketone to form a resonance pair resulting in the inhibition of NO generation, and the inhibition of LPS-induced. Previous studies showed beneficial effect of Justicia gendurussa Burm F (GC) on kidney function. GC demonstrated diuretic activity in rats. Also, it attenuated colitis injury in rats. Justicia gendurussa Burm F displayed antiulcer activity against indomethacin-induced gastric ulcer in rats. Certain flavonoids from Justicia gendurussa Burm F exerted hypolipidaemic activity in rats.

Keywords: Justicia Gendurussa Burm F, Gentamicin, Nephrotoxicity, Anti-oxidative, Anti-inflammatory and Anti-cancer Properties

I. INTRODUCTION:

Gentamicin:

Gentamicin (GM) is an aminoglycoside antibiotic this is very powerful in treating existence threatening gram-bad infections (1). Unfortunately, 30% of sufferers dealt with with GM for extra than 7 days display a few symptoms and symptoms of nephrotoxicity (2-3).

The occurrence of nephrotoxicity from aminoglycosides has multiplied from 2 to 3% in 1969 to 20% with inside the beyond decade. Despite nephrotoxicity and ototoxicity, the aminoglycosides are constantly being utilized in medical exercise due to their bactericidal efficacy, synergism with β-lactam agents, low cost, constrained bacterial resistance, and a post-antibiotic impact (3-4).

However, their efficacy remains counterbalanced through sizable toxicity in particular nephrotoxicity which result in kidney harm through a right away dose-established mechanism (5-6).

High doses (forty mg/kg or extra for gentamicin) are important in animals to unexpectedly result in prolonged cortical necrosis and overt renal dysfunction (7-8).

Nephrotoxicity prompted because of the capsules exert their poisonous outcomes through one or extra not unusualplace pathogenic

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mechanisms that have been stated above. In sure sufferers this drug-precipitated nephrotoxicity has a tendency to be extra not un-usual place and in unique medical situations (9).

II. METHODOLOGY

Table.1: Chemicals

S. No.	Materials	Manufacturers
1.	Gentamicin (100 mg/ml)	Abbott Healthcare Pt. Ltd
2.	EDTA	Fisher Scientific Pt. Ltd, Mumbai
3.	Sodium bicarbonate	Fisher Scientific Pt. Ltd, Mumbai
4	Hydroxylamine hydrochloride	Finar Ltd, Ahmedabad
5	Na-dihydrogen orthophosphate	Fisher Scientific Pt. Ltd, Mumbai
6	Sodium carbonate	Fisher Scientific Pt. Ltd, Mumbai
7	Copper sulphate	Fisher Scientific Pt. Ltd, Mumbai
8	Potassium sodium tartarate	Merck Ltd, Mumbai
9	Potassium iodide	Fisher Scientific Pt. Ltd, Mumbai
10	Nitro blue tetrazolium	Fisher Scientific Pt. Ltd, Mumbai
11	Sodium phosphate dehydrate	Fisher Scientific Pt. Ltd, Mumbai
12	Albumin	Fisher Scientific Pt. Ltd, Mumbai
13	Hydrogen peroxide	Fisher Scientific Pt. Ltd, Mumbai
14	Sodium hydroxide pellets	Fisher Scientific Pt. Ltd, Mumbai

Table.2: Instruments

Instruments	Manufacturers
Semi auto analyser	Recorders & Medicare systems Pvt, Ltd.
Cold centrifuges & Homogenizer	Remi motors Ltd, Mumbai.
Deep freezer	Blue star, Samsung
Micro pipettes	Nichiryo, Japan.
UV Spectrophotometer (UV 1800)	Shimadzu Corporation.
Micro tips, Eppendorf tubes	Tarsons Pvt Ltd, Kolkata.
Soxhlet Apparatus	Gerhardt analytic systems
Micro centrifuge	Beckman
Heating mantle	Shital scientific industries, Mumbai

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Pharmacognostic Study:
Physicochemical Analysis:
Preliminary Phytochemical Study.
Experimental Study.
Source of Data.

Pharmaceutical source:
The part used for this was leaf. The leaf was collected from the garden of TIPER Meerut. Medicine used for research work is kwatha form which was daily prepared at TIPER research Centre as per classical text reference.

Analytical source:
The Pharmacognostic study, Physico-chemical study and the preliminary Phytochemical study was conducted in TIPER Meerut.

Pharmacognostic study:
Collection of sample: Fresh leaves of Vataghni (Justicia gendarussa Burm F.) was collected from herbal garden of Translam institute of pharmaceutical education and research. Meerut.

Place of work: Translam institute of pharmaceutical education and research. Meerut.

Microscopy:
Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars [10].

PHYSICOCHEMICAL ANALYSIS:

Loss on Drying at 105^{o}C:
10 g of sample was placed in tared evaporating dish. It was dried at 105˚C for 5 hours in hot air oven and weighed. The drying was continued until difference between two successive weights was not more than 0.01 after cooling in desiccator. Percentage of moisture was calculated with reference to weight of the sample [10]..

Total Ash:
2 g of sample was incinerated in a tarred platinum crucible at temperature not exceeding 450˚C until carbon free ash is obtained. Percentage of ash was calculated with reference to weight of the sample.

Acid Insoluble Ash:
To the crucible containing total ash, add 25ml of dilute HCl and boil. Collect the insoluble matter on ash less filter paper (Whatmann 41) and wash with hot water until the filtrate is neutral. Transfer the filter paper containing the insoluble matter to the original crucible, dry on a hot plate and ignite to constant weight. Allow the residue to cool in suitable desiccator for 30 mins & weigh without delay. Calculate the content of acid insoluble ash with reference to the air dried drug [11].

Water Soluble Ash:
Boil the ash for 5 min with 25 ml of water; collect insoluble matter on an ash less filter paper, wash with hot water, and ignite for 15 min at a temperature not exceeding 450˚C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water soluble ash with reference to the air-dried sample [11].

Alcohol Soluble Extractive:
Weigh accurately 4 g of the sample in a glass stoppered flask. Add 100 ml of distilled Alcohol (approximately 95%). Shake occasionally for 6hours. Allow to stand for 18 hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105℃ for 6hours, cool in desiccator for 30 minutes and weigh. Calculate the percentage of Alcohol extractable matter of the sample. Repeat the experiment twice, and take the average value [12].

Water Soluble Extractive:
Weigh accurately 4g of the sample in a glass stoppered flask. Add 100 ml of distilled water, shake occasionally for 6hours. Allow to stand for 18hours. Filter rapidly taking care not to lose any solvent. Pipette out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporate to dryness on a water bath. Keep it in an air oven at 105℃ for 6 hours. Cool in a desiccator & weigh. Repeat the experiment twice. Take the average value [12].

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PRELIMINARY PHYTOCHEMICAL TESTS [13]

Table.4.3: Preliminary Phytochemical Analysis

S.No.	Tests	Color if positive	Leaf of Vatagni (Justicia gendarussa Burm F.)
1.	**Alkaloids**		
	Dragendrof's test	Orange precipitate	Light green precipitate
	Wagners test	Red precipitate	Brown precipitate
	Mayers test	Dull white precipitate	Green precipitate
	Hagers test	Yellow precipitate	Yellowish green precipitate
2.	**Steroids**		
	Liebermann- buchard test	Bluish green color	No Bluish green color
	Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	No Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
3.	**Carbohydrate**		
	Molish test	Violet ring	Violet ring
	Fehlings test	Brick red precipitate	Blue color
	Benedicts test	Red precipitate	Bluish green
4.	**Tannin**		
	With $FeCl_3$	Dark blue or green or brown	Green color
5.	**Flavanoids**		
	Shinoda's test	Red to pink	Green color
6.	**Saponins**		
	With $NaHCO_3$	Stable froth	No stable froth
7.	**Triterpenoids**		
	Tin and thionyl chloride test	Red	Green color
8.	**Coumarins**		
	With 2 N NaOH	Yellow	Green color
9.	**Phenols**		
	With alcoholic ferric chloride	Blue to blue black, brown	Green color
10.	**Carboxylic acid**		
	With water and $NaHCO_3$	Brisk effervescence	No effervescence
11.	**Resin**		
	With aqueous acetone	Turbidity	No turbidity
12.	**Quinine**		
	5% NaOH	Pink/purple/red	Green color
13	**Amino acids**		
	Ninhydrine reagent	Purple color	Green color

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Pharmacological Evaluation:

Experimental Animals:

Wistar albino rats (150-200g) of both sexes were purchased from Adita Biosys Private Ltd, Delhi. All the animals were acclimatized for seven days under standard husbandry conditions i.e. room temperature 27± 10ºC, humidity 45-55%, 12 h light and 12 h dark cycle. The animals had free access to standard rodent pellet diet with water; they were housed in polypropylene (32 X 24 X 16 cm) cages. Animals were habituated to laboratory conditions prior to experimental protocol. All the protocols and experiments were conducted in strict compliance according to guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The approval (IAEC/NCP/99/2017) from the Institutional Animal Ethical Committee (IAEC) Translam institute of pharmaceutical education & research. Meerut was taken prior to the experiments.

Acute Toxicity Study:

Animals were doses with both the doses 2000 mg/kg and 4000 mg/kg to check for acute toxicity studies.

Treatment Protocol:

Gentamicin - Induced Nephrotoxicity:

Wistar albino rats (150-200 g) of either sex were used for the study. Animals were divided into 6 groups, each containing 6 animals.

Groups	Treatment
1	Control (5ml of distilled water for 8 days)
2	Gentamicin (100mg/kg/day) was given daily for 8 days through i.p.
3	Low dose (200 mg/kg, p.o.) of ethanolic leaves extract of Justicia gendarussa + gentamicin (100mg/kg, i.p.), respectively for 8 days.
4	High dose (400 mg/kg, p.o.) of ethanolic leaves extract of Justicia gendarussa + gentamicin (100mg/kg, i.p.), respectively for 8 days.
5	Standard drug Cystone (5ml/kg b.w.) + gentamicin (100mg/kg, i.p.), respectively for 8 days.
6	High dose (400 mg/kg, p.o.) of ethanolic leaves extract of Justicia gendarussa for 8 days.

Collection of Urine:

After 8 days, the animals were kept individually in the metabolic cages for the urine sample collection. Volume and pH were measured immediately after collection, 24 hr urine sample was acidified to pH 2 with 5M HCl, was centrifuged at 1500 ×g for 10 min to remove debris and supernatants were stored at −4°C until analyzed[58]. Urine samples were analysed for various biochemical parameters like urea, creatinine, and uric acid [14].

Collection of Blood Samples and Separation of Serum:

After the experimental protocol for 8 days, animals from all 6 groups were anaesthetized with sodium pentobarbital. The blood was drawn from retro orbital plexus in each rat and collected in centrifuge tubes and allowed to coagulate for 30 minutes at 37ºC. The coagulated blood was centrifuged in micro- centrifuge at 2500 rpm for 10 minutes. The freshly prepared serum was used for assay or stored the samples in aliquot at -20ºC which was further used for biochemical estimations of urea, creatinine, and blood urea nitrogen (BUN) [14].

Preparation of Tissue Homogenate:

The rats were sacrificed by the overdose of anaesthesia. The abdomen was dissected opened and kidneys were removed. The isolated kidney was rinsed in ice-cold normal saline to

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remove excess of blood and a 10% w/v homogenate was prepared in 10% chilled phosphate buffer (pH 7.0) (1g of tissue with 10ml of PBS). The tissue was chopped and minced with Teflon homogenizer (on ice) and centrifuged at 3000 rpm for 15 minutes at 4ºC to separate the debris. The collected supernatant was again centrifuged at 5000 rpm for 20 minutes at 4ºC to further break the cell membranes. The obtained supernatant was used for assay or stored at - 20ºC [14].

Evaluation of Physical Parameters: Change in body weight: weight of animal before experiment and afterexperiment.

Serum Analysis

a) Creatinine.
b) Blood urea nitrogen.
c) Urea.

Urine Analysis

a) Creatinine.
b) Uric acid.

Assay Procedure

Addition sequence	Standard	Sample
Working ReagentStandard Sample	1.0 ml100µl -	1.0 ml - 100µl

Calculation

Creatinine concentration (mg/dl) = Δ Abs of sample/ Δ Abs of standard*conc of standard

Estimation of Creatinine:

Clinical Significance: Creatinine is a waste product formed in muscle and forms the energy storage compounds, creatine phosphate. The amount of creatinine produced is fairly constant (unlike urea) and primary a function of muscle mass. It is removed from plasma by glomerular filtration and then excreted in urine without any appreciable reabsorption by the tubules. Creatinine is a useful indicator of renal function. Elevated creatinine level in serum is usually associated with various renal diseases. In the earlier stage of renal disease, creatinine clearance test is a sensitive index of impaired renal function [15].

Principle: Creatinine forms with alkaline picrate (in ratio of 1:1), a coloured creatinine picrate complex containing ionic bounds. The rate of formation of the coloured complex is proportional to the creatinine concentration and measured photometrically at 480-520 nm.

Estimation of Urea:

Clinical Significance: Urea is the major end product of protein metabolism in humans. It constitutes the largest function of the non-protein nitrogen component of the blood. Urea is produced in the liver and excreted through the kidney (90%), gastrointestinal tract or skin. Consequently, the circulating levels of urea depend upon protein intake, protein catabolism and kidney function. Elevated serum urea concentration was observed in impaired kidney function, liver disease, congestive cardiac failure, diabetes, infection, and disease with impaired kidney function [15].

Principle:

The estimation of urea in serum involves the following enzyme catalysed reaction.

Urea + water$\rightarrow 2NH_3 + CO_2$GLDH

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NH_2 + α-KG + ⟶ NADHGLUTAMATE + NAD

Assay Procedure

Addition sequence	Standard	sample
Working ReagentStandard Sample	1.0 ml10µl -	1.0 ml - 10µl

Mix well read variation of absorbance (ΔA) between 30sec and 60 sec.

Calculation

Urea (mg/dl) = ΔA of sample/ΔA of standard*concentration of standard (mg/dl)

Estimation of Uric Acid:

Clinical significance: Uric acid is the end product in purine metabolism; it is removed from plasma by glomerular filtration and then excreted urine without any appreciable reabsorption by tubules. Uric acid is a useful indicator of renal function. Elevated uric acid levels in serum are usually associated various renal diseases. In earlier stage of renal disease, uric acid clearance test is a sensitive index of impaired renal function.

Principle: Uric Acid is converted by urease into allantoin and hydrogen peroxide. The hydrogen peroxide initiates the coupling of 4-aminoantipyrine to 3,5-dichloro-2- hydroxybenzene sulfonic acid (DHBS) to form the chromogen which is measured at 520nm and which is proportional to the amount of hydrogen peroxide generated from uric acid [15].

Assay Procedure:

Addition sequence	Blank	Standard	Sample
Working Reagent Standard Sample	1ml - -	1.0 ml25µl -	1.0 ml - 25µl

ESTIMATION OF ENDOGENOUS ANTIOXIDANT LEVELES IN KIDNEY:

Tissue Preparation: Animals were sacrificed, the kidneys were removed and dissected free from the surrounding fat and connective tissue and immediately weighed. A 10% kidney homogenate was prepared separately with ice-cold saline phosphate buffer (0.05 M, pH 7.4). Homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT), activity using colorimetric assay.

Estimation of Superoxide Dismutase (SOD):

Procedure: Kidney homogenate (0.5ml) was taken and 1 ml of 50mM sodium carbonate, 0.4 ml of 24 µM nitro blue tetrazolium (NBT) & 0.2 ml of 0.1mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1mM hydroxylamine hydrochloride. Zero- time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25°C. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per mg of proteins [15].

Estimation of Catalyse Activity:

Procedure: CAT activity was determined spectrophotometrically. To 1.95ml of 10mM

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H_2O_2 in 60 mM phosphate buffer (pH = 7.0), 0.05 ml of the kidney homogenate was added, and rate of degradation of H_2O_2 was followed at 240 nm/min. CAT content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2.

Histopathological Studies:

Portion of the kidney from all the experimental groups were fixed in 10 % v/v formaldehyde, dehydrated in graded alcohol, cleared in xylene, and then embedded in paraffin. Microtome sections (5µm thick) were prepared from each kidney sample and stained with haematoxylin- eosin (H&E) dye. The six coded slides from each group were examined by a pathologist in a blinded manner.

Statistical Analysis The data were expressed as mean ± SEM. Statistical comparisons were performed by one-way analysis variance (ANOVA) followed by Tukey's post-hoc test using Graph Pad Prism version 5.0, USA. The p value less than 0.05 were considered as statistically significant when compared to control.

III. RESULTS:

Preliminary Phytochemical Evaluation:

S. No.	TESTS	Result
1.	**Carbohydrates**	+
	Molisch's test	+
	Benedict's test	+
	Fehling's test	+
	Barfoed's test	+
2.	**Proteins**	+
	Millon's test	+
	Biuret test	+
3.	**Amino acids**	+
	Ninhydrin's test	+
4.	**Steroids**	+
	Salkowski reaction	+
	Libermann-burchard's Reaction	+
5.	Flavonoids glycosides	+
	Shinoda test	+
	Alkaline test	+
6.	Anthraquinone glycosides	+
	Borntrager's test	+

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7.	Saponin glycosides	-
	Foam test	-
8.	ALKALOIDS	+
	Dragendroff's test	-
	Mayer's test	-
	Hager's test	+
	wagner's test	+
9.	CARDIAC GLYCOSIDES	+
	Legal test	+
	Killer killani test	+
10.	Tannins and phenols	+
	Ferric chloride test	+
	Lead acetate test	+

(+) indicates presence while (-) stands for absence.

Effect Of Ethanolic Justicia Gendurussa Burm F On Blood Urea Nitrogen (Bun): Gentamicin injection resulted in increased BUN levels as compared to normal control (***p< 0.001; $F_{5, 17}$=26.98). Justicia gendurussa Burm F (GC) treatment for 8 days resulted in complete restoration of BUN levels in gentamicin animals. Both the doses i.e 200 mg/kg (Low dose of GC) and 400 mg/kg (High dose of GC) restored BUN values in gentamicin group (***p< 0.001). There was no dose-dependent effect seen with GC on BUN levels. TheBUN levels in Justicia gendurussa Burm F groups were comparable to the standard antioxidant drug cystone. The GC at high dose did not change BUN values in normal animals (p> 0.05).

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S. No	GROUP	Mean ± S.E.M	p value	Number of animals
1.	NC	16.75 ± 0.478	-	4
2.	GEN	146.8 ± 20.07	p < 0.001 vs NC	4
3.	LGC + GEN	46.33 ± 3.756	p< 0.001vs GEN	3
4.	HGC + GEN	62.25 ± 7.983	p< 0.001vs GEN	4
5.	Cystone + GEN	50.75 ± 3.568	p< 0.001vs GEN	4
6.	NC + HGC	15.00 ± 0.408	-	4

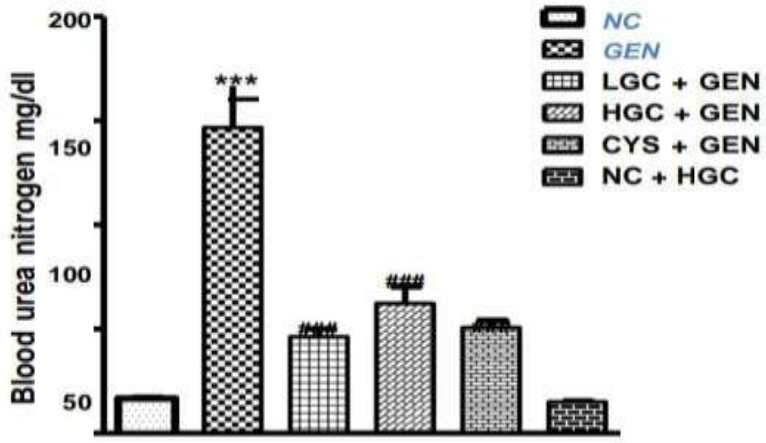

Fig.1: Effect of Justicia gendurussa Burm F on BUN levels.

EFFECT OF ETHANOLIC JUSTICIA GENDURUSSA BURM F ON SERUM UREA:

Gentamicin injection resulted in increased serum urea levels as compared to normal control (***p<0.001, $F_{5,17}$ = 27.66). Justicia gendurussa Burm F (GC) treatment for 8 days resulted in complete restoration of serum urea levels in gentamicin animals. Both the doses i.e. 200 mg/kg (Low dose of GC) and 400 mg/kg (High dose of GC) restored serum urea values in gentamicin group (***p<0.001). There was no dose-dependent effect seen with GC on serum urea levels. The serum urea levels in Justicia gendurussa Burm F groups were comparable to the standard antioxidant drug cystone. The GC at high dose did not change serum urea values in normal animals (p> 0.05).

S. No	GROUP	Mean ± S.E.M	p value	Number ofanimals
1.	NC	36.75 ± 0.854	-	4
2.	GEN	314.8 ± 42.75	p < 0.001 vs NC	4
3.	LGC + GEN	100 ± 7.810	p< 0.001vs GEN	3
4.	HGC + GEN	134.5 ± 17.11	p< 0.001vs GEN	4

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5.	Cystone + GEN	121.5 ± 5.204	p< 0.001vs GEN	4
6.	NC + HGC	31 ± 0.707	-	4

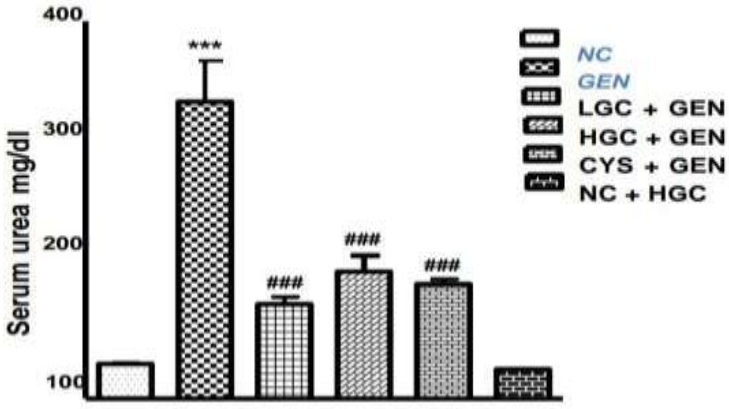

Fig.2: Effect of Justicia gendurussa Burm F on serum urea levels.

EFFECT OF ETHANOLIC JUSTICIA GENDURUSSA BURM F ON SERUM CREATININE:

Gentamicin injection resulted in increased serum creatinine levels as compared to normal control (p 0.01, $F_{5, 18}$ = 6.944). Justicia gendurussa Burm F (GC) treatment for 8 days resulted in restoration of serum creatinine levels in gentamicin animals. Both the doses i.e 200 mg/kg (Low dose of GC) and 400 mg/kg (High dose of GC) restored serum creatinine values in gentamicin group ($^{\#}p< 0.05$ for LGC and $^{\#\#}p< 0.01$ for HGC). There was no dose-dependent effect seen with GC on serum creatinine levels. The serum creatinine levels in Justicia gendurussa Burm F groups were comparable to the standard antioxidant drug cystone. The GC at high dose did not change BUN values in normal animals (p> 0.05).

S. No	GROUP	Mean ± S.E.M	p value	Number ofanimals
1.	NC	0.463 ± 0.030	-	4
2.	GEN	4.450 ± 1.034	p< 0.01 vs NC	4
3.	LGC + GEN	1.630 ± 0.998	p< 0.05 vs GEN	4
4.	HGC + GEN	0.742 ± 0.193	p< 0.01 vs GEN	4
5.	Cystone + GEN	0.726 ± 0.066	p< 0.01 vs GEN	4
6.	NC + HGC	0.349 ± 0.043	-	4

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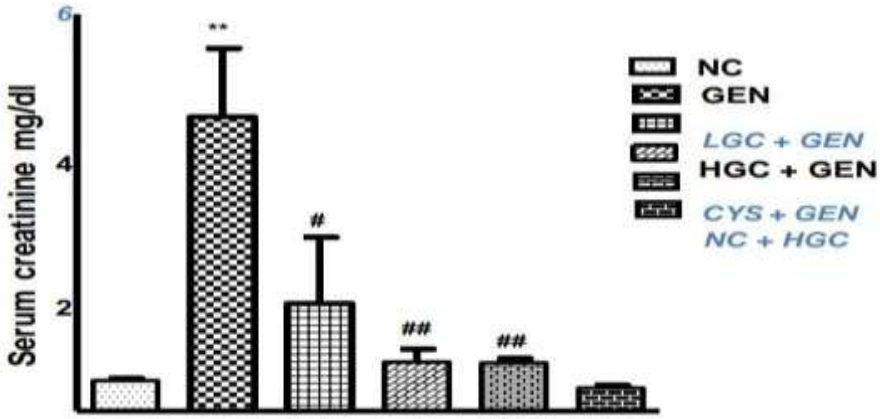

Fig.3: Effect of Justicia gendurussa Burm F on serum creatinine levels.

EFFECT OF ETHANOLIC JUSTICIA GENDURUSSA BURM F ON URINE CREATININE:

We didn't see any changes in urine creatinine values in gentamicin injected animals compared to normal control ($p< 0.5$). One way Anova followed by Tukey's hoc test revealed that there was nosignificant difference among groups in urine creatinine levels ($F_{5,18}=2.352$, $p=0.0826$).

S. No	GROUP	Mean ± S.E.M	p value	N
1.	NC	0.774 ± 0.238	-	4
2.	GEN	0.238 ± 0.017	Ns	4
3.	LGC + GEN	0.695 ± 0.237	Ns	4
4.	HGC + GEN	0.614 ± 0.090	Ns	4
5.	Cystone + GEN	0.480 ± 0.055	Ns	4
6.	NC + GEN	0.270 ± 0.035	-	4

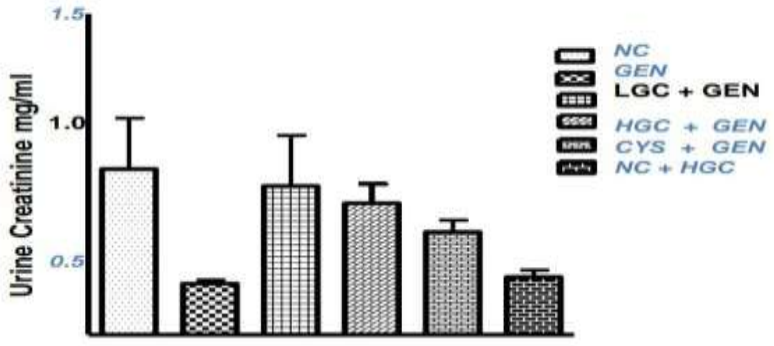

Fig.4: Effect of Justicia gendurussa Burm F on urine creatinine levels.

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EFFECT OF ETHANOLIC JUSTICIA GENDURUSSA BURM F ON URINE URIC ACID:

S. No	GROUP	Mean ± S.E.M	p value	N
1.	Normal	0.281 ± 0.088	-	4
2.	GEN	0.336 ± 0.069	ns	4
3.	LGC + GEN	0.533 ± 0.132	ns	4
4.	HGC + GEN	0.412 ± 0.033	ns	4
5.	Cystone + GEN	0.443 ± 0.018	ns	4
6.	HGC without GEN	0.872 ± 0.032	-	4

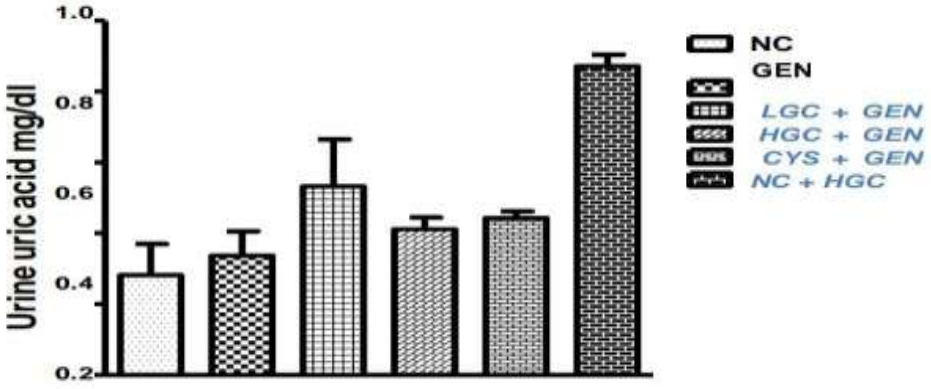

Fig.5: Effect of Justicia gendurussa Burm F on urine uric acid levels.

EFFECT OF ETHANOLIC JUSTICIA GENDURUSSA BURM F ON URINE UREA:

S.No	GROUP	Mean ± S.E.M	P value	N
1.	Normal	35 ± 5.888	-	4
2.	GEN	12 ± 0.912	Ns	4
3.	LGC + GEN	57 ± 13.39	Ns	4
4.	HGC + GEN	59 ± 4.435	Ns	4
5.	Cystone + GEN	29 ± 2.041	Ns	4
6.	HGC without GEN	23.75 ± 1.493	-	4

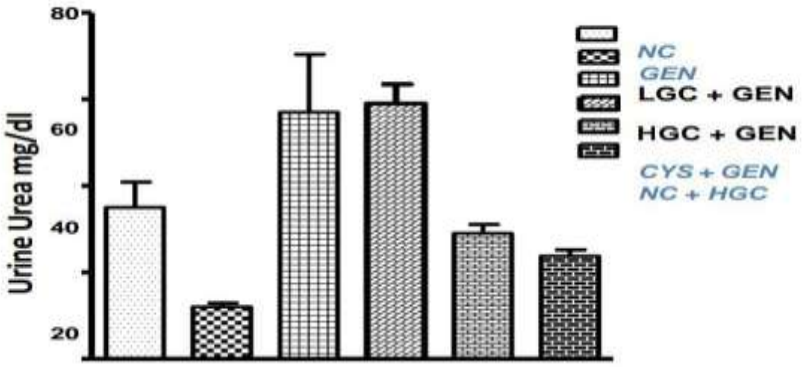

Fig.6: Effect of Justicia gendurussa Burm F on urine urea levels.

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Effect of EGC on body weight gain in gentamicin induced nephrotoxicity in rats: We measured weight on 1st day and on 8th days after treatment for 8th days. Gentamicin injected rats showed decreased in body weight gain as per normal control. GC treated rats showed a slight increased in body weight gain, but is not comparable to normal control. The standard drug Cystone treated animal showed significantly enhanced body weight gain.

GROUPS	Mean ± SEM	% Change in body weight gain
NC	7.980 ± 0.429	7.226
GEN	-5.416 ± 1.817	-4.234
LGC + GEN	1.902 ± 0.157	1.789
HGC + GEN	3.259 ± 1.005	2.621
CYS + GEN	12.04 ± 5.321	7.504
HGC + NC	4.574 ± 1.446	5.497

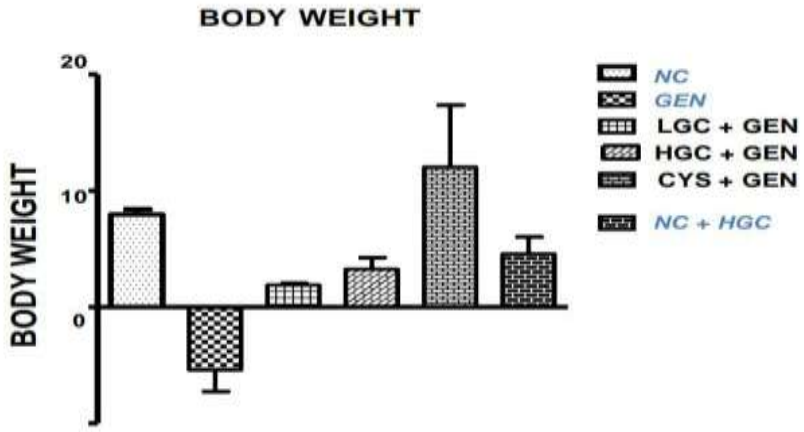

Fig.7: Effect of Justicia gendurussa Burm F on body weight gain, gentamicin injection reduced body weight gain.

Effect of Justicia gendurussa Burm F on CAT:

Catalase activity decreased in gentamicin compared to normal. Both the doses 200 mg/kg and 400 mg/kg decreased compared to gentamicin. The standard drug Cystone was comparable with gentamicin and the normal control group shows decreased catalase activity when compared with normal.

Groups	Mean	Standard Error	N
NC	22.72777308	4.936024273	6
GEN	13.77287303	6.886436513	4
LGC + GEN	1.766494722	0.74632209	4
HGC + GEN	2.862663858	0.923489281	5
CYS + GEN	11.34257488	3.919558251	6
NC + HGC	5.773872543	1.677951581	3

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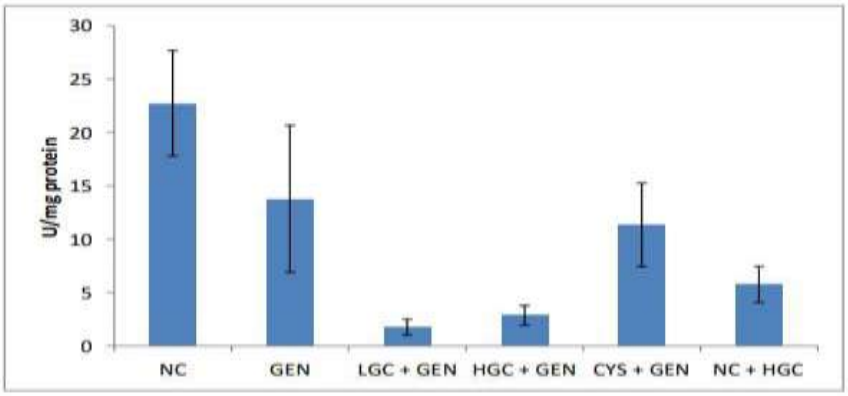

Figure R8: Effect of *Justicia gendurussa Burm F* on catalase. Data expressed as Mean ± SEM. NC -Normal control; GEN - Gentamicin injected; LGC + GEN - Low dose *Justicia gendurussa Burm F* + Gentamicin; HGC + GEN - High dose *Justicia gendurussa Burm F* + Gentamicin; Cystone + GEN - Cystone + Gentamicin; NC + HGC - High dose *Justicia gendurussa Burm F* in normal animals.

EFFECT OF JUSTICIA GENDURUSSA BURN F ON SOD:

The SOD activity was inhibited in Gentamicin when compared with normal. Both the doses 200mg/kg and 400 mg/kg showed decreased inhibition when compared to Gentamicin. The standarddrug Cystone showed decreased inhibition when compared to Gentamicin. The normal control showed increased inhibition compared to normal.

Groups	**% inhibition**
GEN	19.58564289
LGC + GEN	-43.12938816
HGC + GEN	-38.99121607
CYS + GEN	-54.98617063
NC + HGC	18.1190368

ACUTE TOXICITY STUDY:

The animals showed positive response for both the doses 200 mg/kg and 400 mg/kg, after administration of both the doses and observed for 48 hours. EFGCextract did not cause any harm to the animlas.

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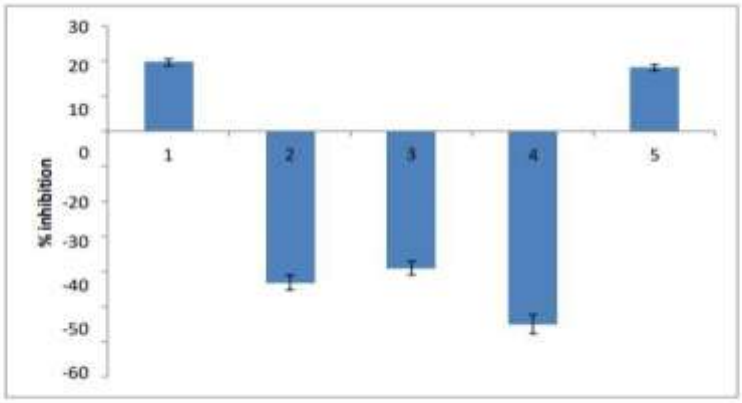

Figure R9: Effect of *Justicia gendurussa Burm F* on SOD. Data expressed as Mean ± SEM. NC - Normal control; GEN - Gentamicin injected; LGC + GEN - Low dose *Justicia gendurussa Burm F* + Gentamicin; HGC + GEN - High dose *Justicia gendurussa Burm F* + Gentamicin; Cystone + GEN - Cystone + Gentamicin; NC + HGC - High dose *Justicia gendurussa Burm F* in normal animals.

HISTOPATHOLOGY REPORTS
G.1.1 – Control
Renal archtitecture - Preserved
Glomeruli – Normocellular with intact bowmen's space (**Fig.10)**
Features suggestive of Normal Renal Morphology
Tubule – Intact with normal tubular epithelium and intra tubular space (**Fig.11)** Blood vessels – Intact
Interstitium - Intact

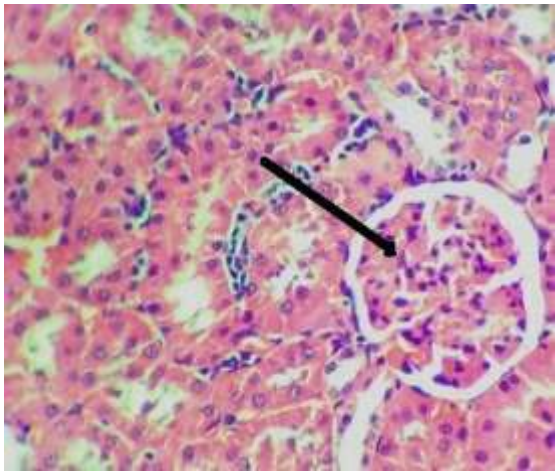

Fig.10. H&E 400X

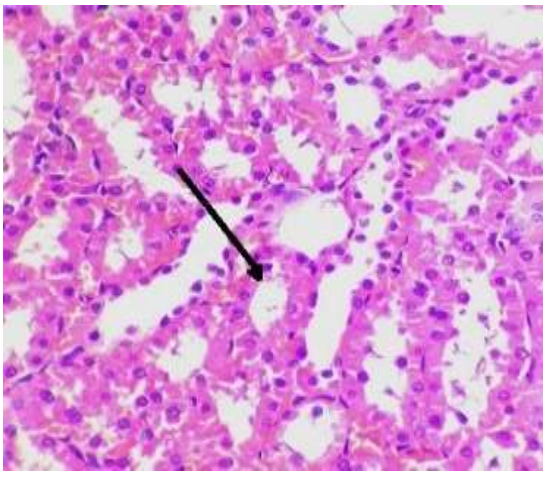

Fig.12. H&E 400X

HISTOPATHOLOGY REPORTS:
Gentamicin (100mg/kg)
Renal archtitecture - Preserved
Glomeruli – Mild hypercellularity with intact bowmens space
Tubule – Dilated, with Necrosis and loss of tubular epithelium. (**Fig.13)**
Many of the tubules show amorphous eosinophillic debris in tubular space. (**Fig.14)**

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Tubular epithelial cells show vacuolization and cytoplasmic inclusionsBlood vessels - Intact

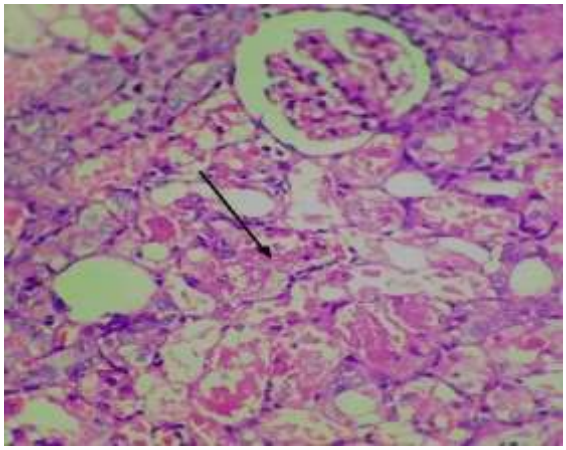

Fig.13 H&E 400X

HISTOPATHOLOGY REPORTS:
High dose of Justicia gendurussa Burm F (400 mg/kg)
Renal archtitecture - Preserved

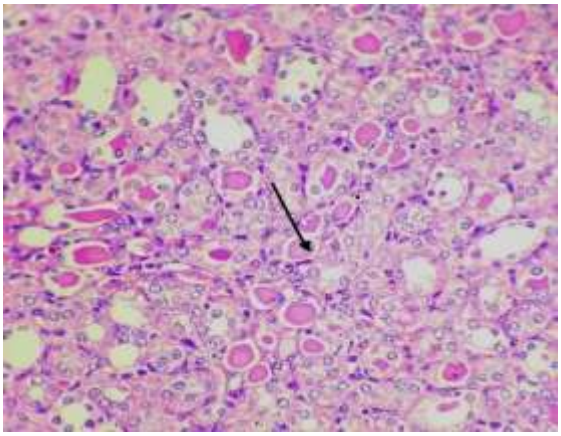

Fig.14 H&E 400X

Glomeruli – Normocellular with intact bowmen's space
Tubule – Intact with normal tubular epithelium and intra tubular spaceBlood vessels - Intact
Interstitium – Intact

Fig.15 H&E 400X

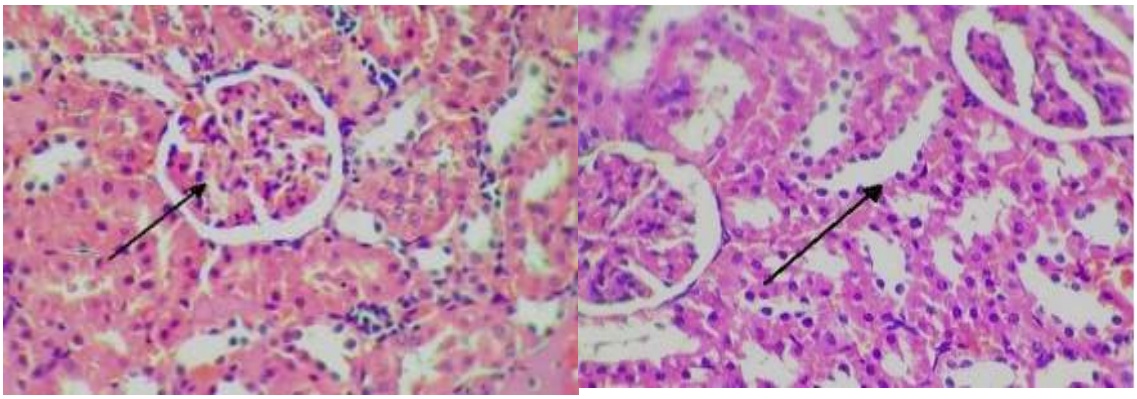

Fig.16 H&E 400X

HISTOPATHOLOGY REPORTS
G.7.1 – Low dose Justicia gendurussa Burm F (200mg/kg) + Gentamicin (100 mg/kg)
Renal archtitecture - Preserved

Interstitium- Intact Features suggestive of Tubular Necrosis Secondary to Drug Nephrotoxicity.

Glomeruli – Normocellular with intact bowmens space (Fig.17a arrow)
Tubule – Almost Intact with normal tubular epithelium and intra tubular space (Fig.18 arrow)
Blood vessels - Intact

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Interstitium - Intact

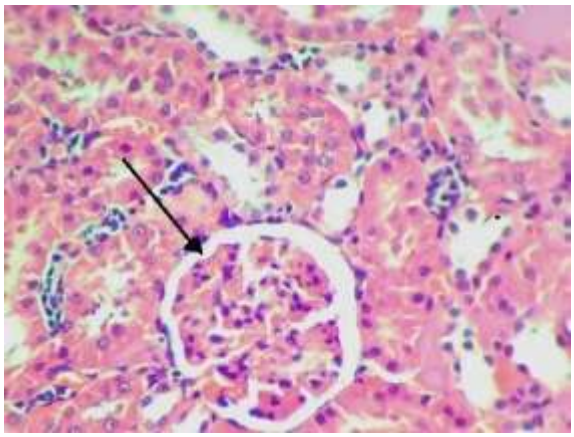

Fig.17 H&E 400X

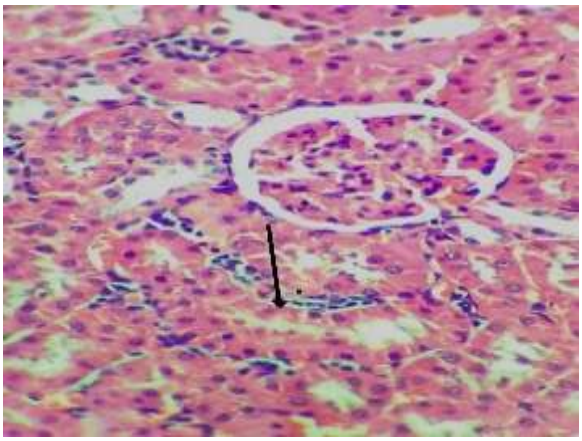

Fig.18 H&E 400X

HISTOPATHOLOGY REPORTS
High dose Justicia gendurussa Burm F (400mg/kg) + Gentamicin (100 mg/kg)
Renal archtitecture - Preserved
Glomeruli – Normocellular with intact bowmens space (Fig.19 a arrow)
Tubule – Almost Intact with normal tubular epithelium and intra tubular space (Fig.20 arrow)
Blood vessels – Intact
Interstitium - Intact

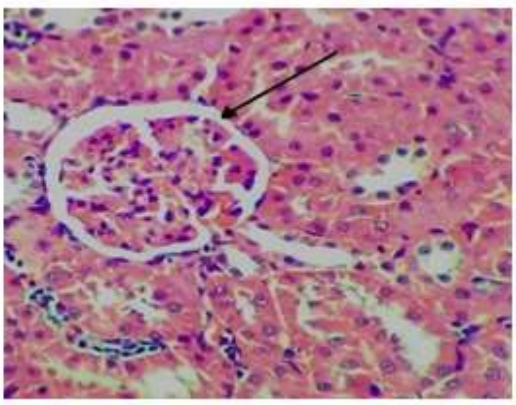

Fig.19 H&E 400X

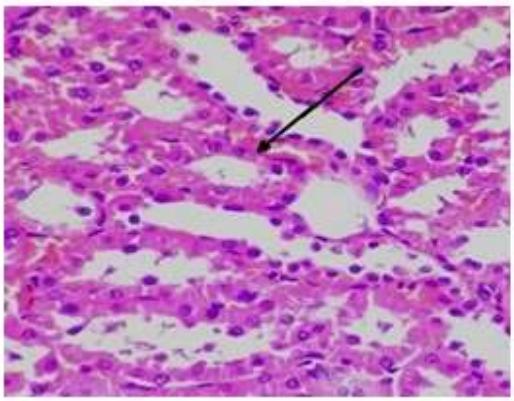

Fig.20 H&E 400X

HISTOPATHOLOGY REPORTS:
Standard drug Cystone + Gentamicin (100 mg/kg)
Renal architecture - Preserved
Glomeruli – Normocellular with intact bowmen's space
Tubule – Almost Intact with normal tubular epithelium and intra tubular spaceBlood vessels - Intact
Interstitium – Intact

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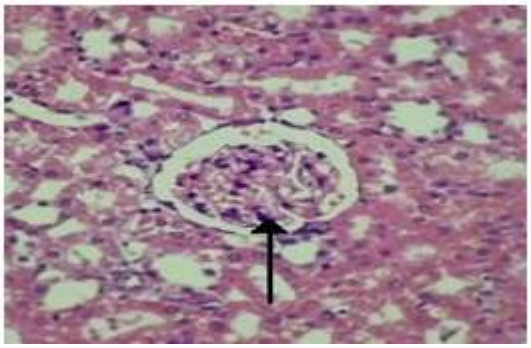

Fig.21 H&E 400X

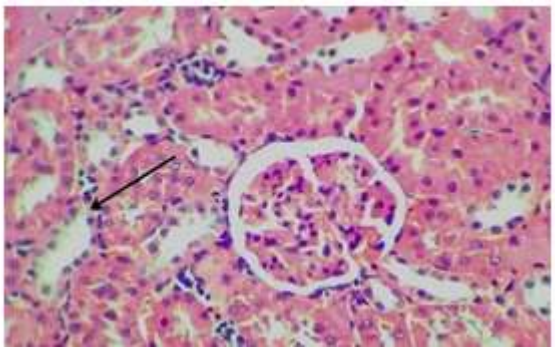

Fig.22 H&E 400X

IV. DISCUSSION

In the current study, simultaneous treatment with ethanolic leaves extracts of Justicia gendurussa Burm F provided a marked functional and histological protection against acute renal damage in rats treated with gentamicin. Increase in blood urea nitrogen, serum urea and s erum creatinine induced by gentamicin was prevented by ethanolic leaves extracts of Justicia gendurussa Burm F. This study revealed that orally-administered ethanolic leaves extracts of Justicia gendurussa Burm F had a significant and dose dependent protective effect in gentamicin-induced nephrotoxicity in rats. A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models. A significant increase in lipid peroxidation products malondialdehyde (MDA) in gentamicin injected rats, suggesting the involvement of oxidative stress. A role of lipid peroxidation in gentamicin-induced acute renal failure has also been described in previous studies. Moreover, pretreatment of rats with hydroxyl radical scavengers has been shown to protect against gentamicin induced acute renal failure. Gentamicin is known to decrease the activities of catalase, and the level of SOD. Garcinol is the main medicinal component of the dried fruit rind of Justicia gendurussa Burm F. In vitro studies of garcinol revealed its potential therapeutic effects, such as its anti-oxidative, anti- inflammatory and anti-cancer properties. Similarly, in vivo studies in animal models also demonstrated the efficacy of garcinol for the treatment of various inflammatory and cancerous conditions. Garcinol has been proposed to react with peroxyl radicals by a single electrontransfer followed by deprotonation from the hydroxyl group of the enolized 1, 3-diketone to form a resonance pair resulting in the inhibition of NO generation, and the inhibition of LPS-induced. Previous studies showed beneficial effect of Justicia gendurussa Burm F (GC) on kidney function. GC demonstrated diuretic activity in rats. Also, it attenuated colitis injury in rats. Justicia gendurussa Burm F displayed antiulcer activity against indomethacin-induced gastric ulcer in rats. Certain flavonoids from Justicia gendurussa Burm F exerted hypolipidaemic activity in rats. Earlier studies reported the nephroprotective activity of different herbal drugs in animals. Bauhinia tomentosa Linn showed nephroprotective effect against cisplatin-induced renal damage. Hydroalcoholic extract of Artemisia arborescens exhibited nephroprotectant and antioxidant properties against oestroprogestative-induced kidney damages in rats. Herbs Eclipta alba (L.) Hassk, Bilvādi agada. Aloe vera and Croton zambesicus protected nephrons in gentamicin-induced nephrotoxicity in rats. Treatment with the aqueous extract of Carica papaya seeds resulted in nephroprotectant activity in paracetamol-induced nephrotoxicity in male Wistar rats. Therefore, it is reasonable to assume that the nephron-protection shown by garcinol in gentamicin-induced nephrotoxicity is mediated through its potent antioxidant effects. The antioxidant activity of garcinol is contributed to its nephron-protective effect by inhibiting gentamicin-induced lipid peroxidation.

V. CONCLUSION

In conclusion, the prevailing have a look at found out the nephroprotectant outcomes of ethanolic leaves extract of Justicia gendurussa Burm F in gentamicin version of nephrotoxicity. The protectant impact is related to recuperation of urea, blood urea nitrogen and creatinine tiers in serum. Also, Justicia gendurussa Burm F indicates anti-oxidant hobby through restoring superoxide dismutase (SOD) and catalase hobby in gentamicin injected rats. This confirms loose radical scavenging outcomes of Justicia gendurussa Burm

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F. The purposeful recuperation is related to entire restoration of morphological modifications in nephron. These useful outcomes of Justicia gendurussa Burm F can be beneficial to deal with kidney harm in distinctive conditions. The drug may be used as an adjuvant remedy for human beings laid low with nephropathies.

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