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Assessment on Nephroprotective Activity of *Cassia Auriculata Linn* on Gentamicin Induced Renal Toxicities in Albino Rats

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***Abstract*: The main objective of our research study is to evaluate nephroprotective potentials of *cassia auriculata Linn* leaf extract against nephrotoxicity. Nephroprotective activity of *Cassia auriculata Linn* (100 mg/kg P.O) + (80mg/kg/day) I.P of gentamicin for 9 days. In this study the standard drug cystone (2ml/kg) and test drug *Cassia auriculata Linn* (100 mg/kg P.O) will be continued to 19th days. After 19th day of last dose animals will be sacrificed under euthanasia and kidney will be removed for histopathological evaluation. The tissue was fixed saline dehydrated with 100% of ethanol solution and embedded in paraffin. It was then processed into 4-5m thick section stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X). All the slides show kidney tissue consisting of cortex and medulla. It also contains tubules in various shapes like proximal convoluted tubule and distal convoluted tubule. From this study, it is clear that the selected medicinal plant plays a prominent role against various diseases. The results of this study indicate that extracts of plant *cassia auriculata linn* does not show any nephrotoxicity and have good potentials for use in kidney damage.**

***Keywords*: Nephroprotective, Nephrotoxicity, Gentamicin, Histopathological evaluation.**

1. Introduction

The Kidneys are the most vital organ in the human body which are involved in the excretion of metabolic substance and waste from the body. (Renal disease are a major problem globally and renal damage is very common medicines which includes antibiotics and environmental toxins significantly modifies the functions and structure of various tissues as well as intestine, heart, kidney, and liver. (Newman T, Biggers, Medical News Today. 2018).

Gentamicin is the one of the most widely used aminoglycoside antibiotic against the urinary tract and abdomen's infections. Although, ototoxicity and nephrotoxicity are still the biggest unfavourable reactions of drug to effectively using it for medical purposes in long-term. (Abhijeet Lakhera, Aditya Ganesh Purkar, Divya Nazneen Dubey et al., Interdisciplinary toxicology, 2015).

Various functional, metabolic and morphological alterations in kidney is caused by the gentamicin as well as gentamicin nephrotoxicity's intensity is linked with its accumulation in convoluted tubules of renal proximal that results in the tubular necrosis. (Annie Shirwai Kar, Deepti Issac, S. Malini et al., Pharm. Sci. & Res. 2016, Vol. 8(9)).

Nephrotoxicity is a poisonous effect due to drugs and its overdosage on the kidneys. A number of antibiotics including penicillin, cephalosporins, tetracyclines as well as aminoglycosides and sulfonamides, are potential nephron toxicants. The drug induced nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate. (Aparna Rama Laxmi Devi. M, Yaso Deepika. M, Nagaraju, Prasad. K et al., IOSR Journal of Pharmacy. 2012;2(2)).

Gentamicin is an antibiotic that exhibits a broad spectrum of activity and is particularly valuable in severe species. Its use is restricted due to development of ototoxicity and nephrotoxicity. At physiological pH, the drug is highly charged and water soluble, and therefore, it is practically unable to diffuse through biologic membranes. Nephrotoxicity has been related to a selective accumulation of gentamicin in the renal cortex. (Hussain T. et al., Asian Pac J. Trop Med (2012)).

Approximately 8% to 26% of patients who receive aminoglycosides for more than 7-10 days develop mild reversible impairment. It usually presents as gradually worsening non oliguric renal failure and sometimes acute tubular necrosis may occur.

There are three analytical methods to detect Nephroprotective activity.

1. *Serum Analysis:* The collected blood samples were centrifuged for 10 min at 10,000rpm as well as the serum samples were subjected to estimation of biochemical parameters such as BUN (body urea nitrogen), uric acid, creatinine, and electrolytes (chlorides, potassium and sodium).
2. *Kidney Homogenate Analysis:* The isolated kidneys were homogenized with homogenizer. The kidney homogenates were subjected to in-vivo antioxidant study using Lipid peroxidation [LPO] and glutathione

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estimation respectively.

3. Histopathological Studies: The animals from all the respective groups were euthanized by using CO2 chamber at the end of the study followed by the isolation of kidneys. The slides were prepared by staining with eosin and hematoxylin and observed under electron microscope. (Shimmi SC, Jahan N, Baqi N, Rahman Z et al., J Enam Med Col. 2014;4(1):26– 30).

2. Materials and Methods

A. Experimental Design

Healthy and well grown leaves of selected plant Cassia auriculata Linn were collected from the area Alaganeri during the month of February 2021 and then they were identified and kept in the herbarium sheet with a specimen identification number.

Extraction was done in the following manner, The leaves were dried in a clean dry shade for 7 days, after 7 days its coarsely grinded mixer and immediately brought to the laboratory in a clean container.

B. Preparation of Plant Extraction

The plant extraction was done by Maceration method, The whole/coarsely powdered crude drug is placed in a stoppered container with the solvent.

Allow to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter gets dissolved.

The mixture then is strained, the marc (the damp solid material) is pressed. The combined liquids are clarified by filtration or decantation after standing. This method is best suitable for use in case of the thermolabile drugs.

C. Phyto-Chemical Analysis

The Aqueous extract of Cassia auriculata Linn was subjected to phytochemical analysis find out the presence and absence of phytochemical constituents. The phytochemical tests employed for alkaloids, flavonoids, glycosides, amino acid, carbohydrate, tannins and terpenoids.

D. Nephroprotective Activity

In this nephroprotective activity we have selected 16 albino wistar male rats 2-3 months (150- 250gm). The animals were kept in the polypropylene cages with an optimum supply of food (pellets) and drinking water. All the experimental work on animals was carried out according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and as per the Animal Ethics guidelines of the institution. (SARPC/IAEC/17/2021)

All the animals were acclimatized for a week before the commencement of the experiment.

The animals will be randomly prearranged into FOUR groups of four animals as shown in table 1.

In this study the standard drug cystone (2ml/kg) and test drug *Cassia auriculata Linn* (100 mg/kg P.O) will be continued to 19th days. After 19th day of last dose animals will be sacrificed under euthanasia and kidney will be removed for histopathological evaluation.

E. Histopathological Studies of Rat Kidneys

Kidneys of sacrificed animals were identified and carefully dissected out for histopathological studies after rinsing in normal saline sections were taken from each harvested kidney. The tissue was fixed -saline dehydrated with 100% of ethanol solution and embedded in paraffin. It was then processed into 4-5m thick section stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X).

1) Procedure and Interpretation

The tissue was kept in 10% formalin for 48 hrs.

Table 2
Processing of tissues

No.	Processing solution	Time
1	70% Alcohol	30 minutes
2	90% Alcohol	30 minutes
3	Absolute Alcohol I	1 hour
4	Absolute Alcohol II	1 hour
5	Absolute Alcohol III	1 hour
6	Xylene I	45 minutes
7	Xylene II	45 minutes
8	Paraffin Wax I	30 minutes
9	Paraffin Wax II	3-4 hours

Blocking or embedding the tissue was done to transfer the tissue from the final wax bath to a mold filled with molten paraffin wax.

Thin sections of tissues block of 4 microns were cut with the help of microtome. The tissue sections are floated in water bath of temperature 50º- 52º and then taken in microscopic slides.

Table 3
H & E staining

No.	Staining solution	Time
1	Xylene I	5 minutes
2	Xylene II	5 minutes
3	Xylene III	10 minutes
4	100% Alcohol	2 minutes
5	70% Alcohol	2 minutes
6	50% Alcohol	2 minutes
8	Running water	2 minutes
8	Hematoxylin	2 minutes
9	Running water	1 minutes
10	Acid alcohol	2 dips
12	Running tap water	10 minutes
13	Eosin	30-60 sec
14	90% alcohol	1 dip
15	100% alcohol	2 mins
16	Xylene	5mins

Coverslip was placed in the slide and the prepared slides were seen under the LX-500 LED trinocular Research microscope (Labomed) and images were taken with MiaCam

Table 1

Group- I	Control- will be received 0.1 ml normal saline for 9 days through orally.
Group-II	Induced control- will be received (80mg/kg/day) I.P of gentamicin for 9 days.
Group-III	Standard- will be received Cystone drug (2ml/kg) + 80mg/kg/day I.P of gentamicin for 9 days.
Group-IV	Test - will be received *Cassia auriculata Linn* (100 mg/kg P.O) + (80mg/kg/day) I.P of gentamicin for 9 days.

CMOS AR 6pro microscope camera connected to image AR pro software.

F. Histopathology of Control Rat Kidney

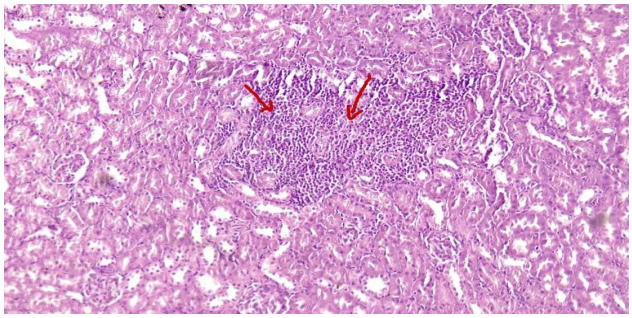

Fig. 1. 100x-inflammatory infiltrate (2) – marking

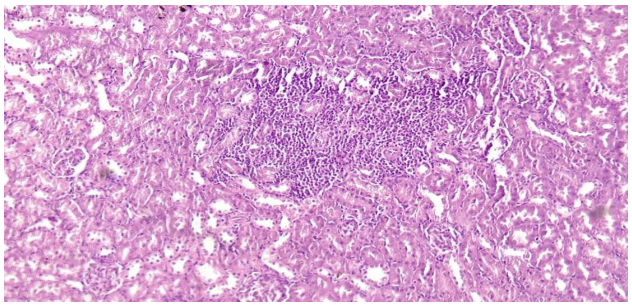

Fig. 2. 100x-inflammatory infiltrate (2)

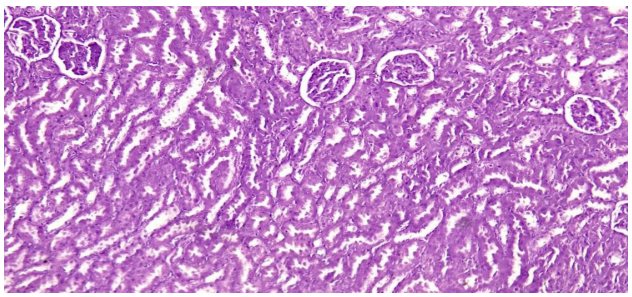

Fig. 3. 100x-normal tubules

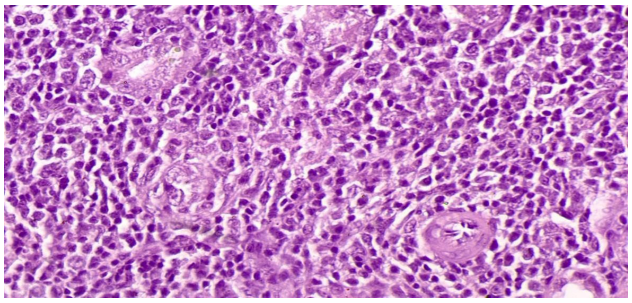

Fig. 4. 400x-chronic inflammatory infiltrate (2)

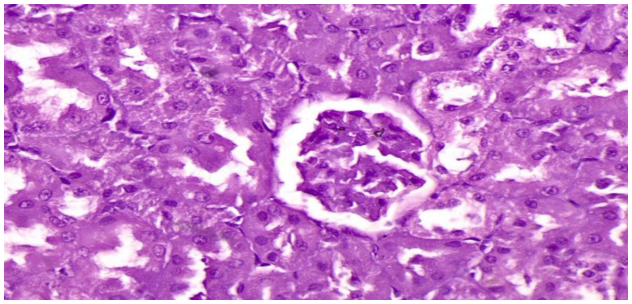

Fig. 5. 400x-normal tubules

G. Histopathology of Induced Control Rat Kidney

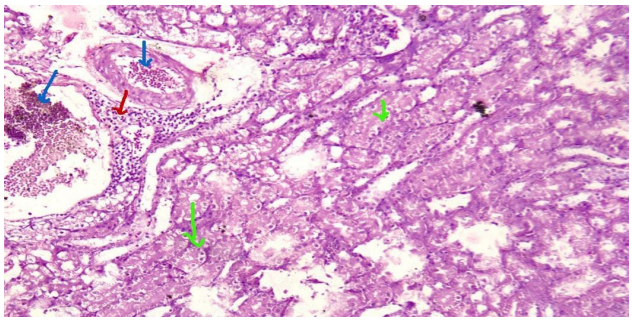

Fig. 6. 100x-inflammatory infiltrate (red), congested blood vessels (blue), degenerated tubules (green)-marking

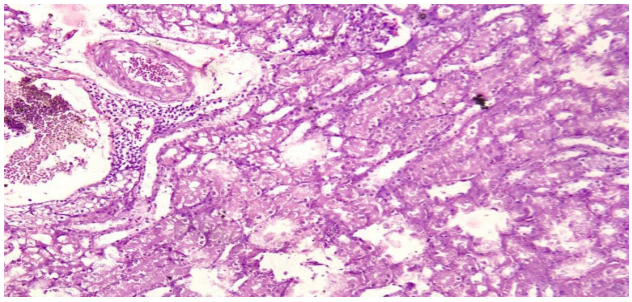

Fig. 7. 100x-inflammatory infiltrate, congested blood vessels, degenerated tubules

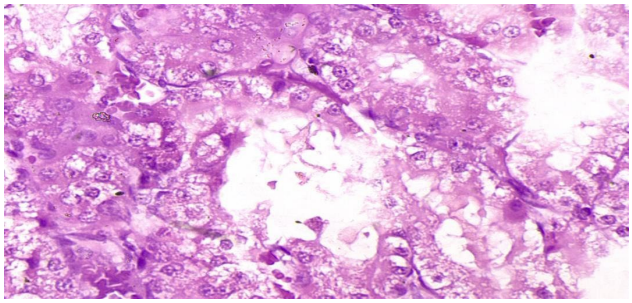

Fig. 8. 400x degenerated tubules

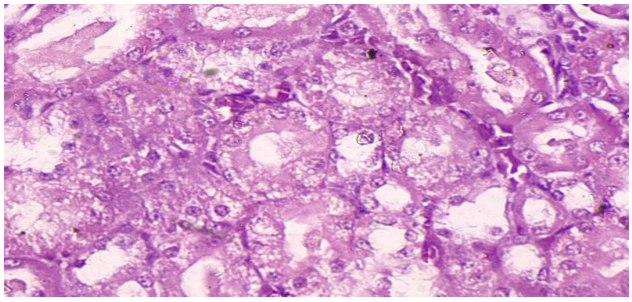

Fig. 9. 400x-degenerated tubules

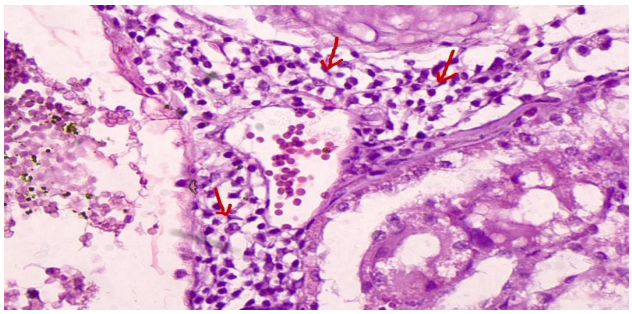

Fig. 10. 400x-inflammatory infiltrate

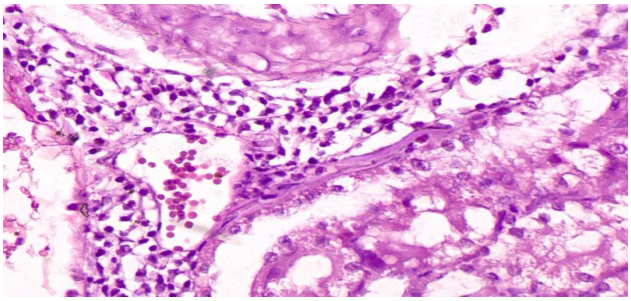

Fig. 11. 400x-inflammatory infiltrate-marking

H. Histopathology of Test Rat Kidney

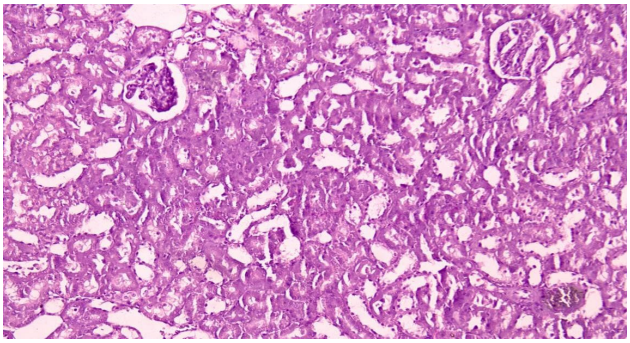

Fig. 12. 100x-areas without degenerated tubules

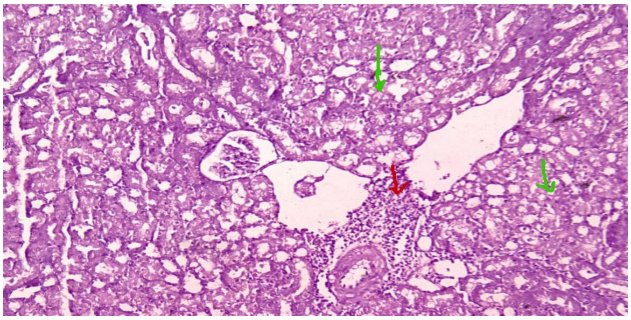

Fig. 13. 100x-inflammatory infiltrate (red) and degenerated tubules (green)-marking

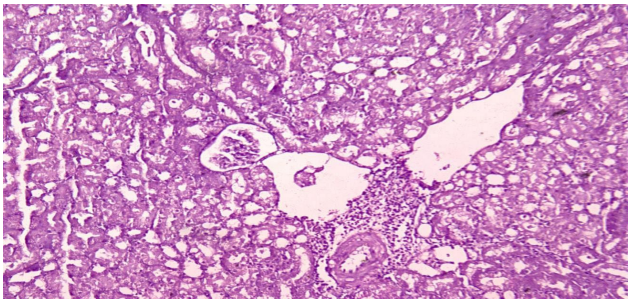

Fig. 14. 100x-inflammatory infiltrate and degenerated tubules

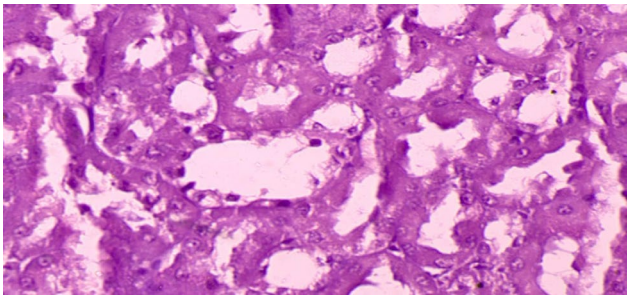

Fig. 15. 400x-areas without degenerated tubules

I. Histopathology of Standard Rat Kidney

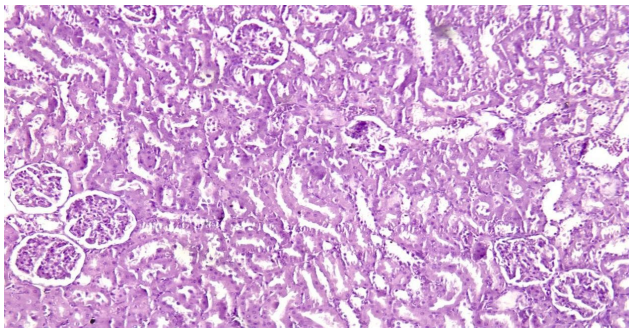

Fig. 16. 100x-areas without degenerated tubules

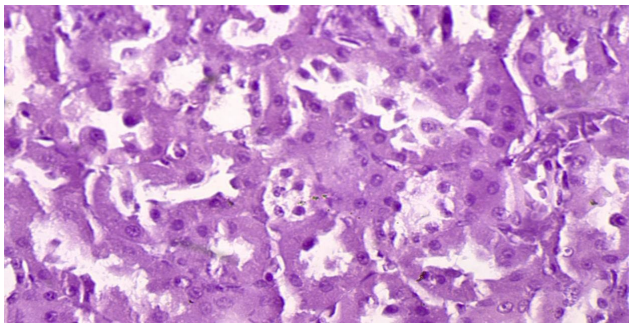

Fig. 17. 400x-areas without degenerated tubules

3. Result and Discussion

A. Report

Table 4
Kidney changes seen

Slide No.	Necrosis	Inflammation	Degeneration
4A-control	-	-	-
4A-control (2)	-	+	-
4B-induced control	-	+	+++
4B-induced control (2)	-	-	-
4C test	-	+	+
4C test (2)	-	-	+++
4D standard	-	-	++
4D standard (2)	-	-	+

- Nil, + mild, ++ moderate, +++ severe

Grading for degeneration:

- No degenerated tubules
+ <10% degenerated tubules
++10-25% degenerated tubules
+++25-50% degenerated tubules
++++ >50% degenerated tubules

All the slides show kidney tissue consisting of cortex and medulla. The cortex consists of circular structures called corpuscles. It also contains tubules in various shapes like proximal convoluted tubule and distal convoluted tubule.

4A-control: No degeneration or necrosis. Inflammatory infiltrate seen in one slide.

4B-induced control: Many tubules in the corticomedullary region show degeneration with pale vacuolated cytoplasm and pyknotic nuclei, with sloughing of cell remnants in lumen. Inflammatory infiltrate and congested blood vessels seen

4C-Test: Small area in the cortico medullary region shows degenerated tubules. Compared with 2B induced control group, there is reduction in degeneration in one slide. No inflammation

or necrosis seen.

2D standard: Small area in the cortico medullary region shows degenerated tubules. Compared with 2B induced control group, there is reduction in degeneration. No inflammation or necrosis seen.

4. Conclusion

The Aqueous extract of selected plant protected gentamycin–induced renal toxicity in rats. From this study, it is clear that the selected medicinal plant plays a prominent role against various diseases. The medicinal plant extracts have been reported for its significant nephroprotective activity in animal models. The results of this study indicate that extracts of plant *cassia auriculata linn* have good potentials for use in kidney damage.

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