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# RENO-PROTECTIVE POTENTIAL OF ETHANOLIC HERBAL EXTRACTS AGAINST GENTAMICIN CHALLENGED ALBINO WISTAR RATS

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## ABSTRACT
Renotoxicity could be a usual disorder characterized as a fast decrease in kidney work coming about in irregular maintenance of Sr creatinine and blood urea, which should be excreted. There are usual chemical operators to treat intense renal disappointment. The dried roots and the entire plant of Clausena anisata and Oxalis corniculata were extracted by ethanol. the ethanolic extricate appeared factually critical renoprotective action. The plant extricate demonstrated to have renoprotective possibilities since of its known flavonoid and antioxidant character. There may be a opportunity for encourage examination on the histo pathology and medical ponders that are essential to explain the dynamic phytochemical constituents with powerful reno protective activity. The Gentamicin induced rats appeared lifted levels of Sr Blood Urea Nitrogen (BUN) and lipidperoxidation parameter like malon di aldehyde (MDA) which was essentially diminished with therapy of EECA AND EEOC, which demonstrates its Renoprotective movement.

**KEYWORDS:** renoprotective agents, Clausena anisata and Oxalis corniculata.

## INTRODUCTION
The Wellbeing Organization gauges that almost 80% of populace living within the creating nations depends on conventional medications for their essential wellbeing and careneeds. In nearly every conventional framework of solutions, the restorative plants plays majorpart and forms the spine. An expansive body of prove has gathered to appear likely of restorative plants utilized in different conventional frameworks. Within final hardly any long time more than 13K plants have been examined for the different infections & afflictions all over the world. Home grown medication are considered and utilize for restorative properties of plants. Plants have the capacity to synthesis a wide assortment of chemical mioeties that are utilized to performs vital organic capacities. Many of the phytochemical advantageous impacts devoured by man, they are utilized viably treat illness. At slightest 12K such compound were separated so distant; a numb assessed to be <10% of the entire. Chemical components in plants intervened their impacts on the body through forms indistinguishable to which as of now well caught on for the chemical components in basic drugs; in this way home synthesized solutions don't vary significantly from ordinary drugs in terms of how they work. This empowers home synthesized drugs to be as feasible as corrective solutions.

## AIM
To evaluate the phytochemical constituents & renoprotective potential of ethanolic extricates of domestic developed plants that are careful for the renoprotective activity in gentamicin started renotoxicity in rats.

## OBJECTIVES
1) Evaluation of renoprotective action by planning and extraction of ethanolic extricates of home grown plants.
2) Phytochemical examination of home grown plant constituents.
3) Acute poisonous quality of ethanolic extracts.
4) To assess the renoprotective movement of the home grown plant extricate in gentamicin initiated renotoxicity

in rats by assessing the biochemical parameters like changes in body weight & kidney weight, serum creatinine, BUN beside antioxidant markers MDA and glutathione.

## MATERIALS AND METHODS

1) **Collection & Authentication of plant material**: The dried roots and the entire plant of Clausena anisata and Oxalis corniculata were collected individually. The plants were taxonomically recognized and authentified by Dr. K.Madhava Shetty, Partner Teacher of Botany, Division of Pharmacognosy, SriVenkateshwara College, Tirupathii

2) **Preparation of plant extract**: The plant extraction is done by maceration prepare. The dried takes off were decreased to coarse powder and after that made into fine powder taken after by maceration to get the plant extricate.

**Maceration**: This mode of action accounts for installation of herbal dusts in porcelain decanter adjacent to ethanol (1:2ratio) angled for 7 days at room climate, accompanied by periodic folding. The jar is jacketed with foil for arrest of ethanol escape. Bulk from jar is drained following ethanol pass until semisolid matter is procured.

3) **Chemicals:** next are requisite for the test-

- Ethanol- for construction of herbal pluck outs.
- Gentamicin-for triggering renotoxicity
- Vit E + Vit C – as standard care
- Tween 20- emulsifying medium

4) **Preparatory phytochemical presentation:** Preliminary phytochemical screening will be done to identify chemical constituents such as alkaloids, flavonoids, glycosides, terpenoids, sterols, polyphenols, etc by using standard methods described in practical Pharmacognosy text book by kokate. All the chemicals & reagents used will be of analytical graded.[1]

5) **Research animals:** The experimental research will be conducted at Shadan Institute of Medical Sciences(SIMS), Peerancheru, HYD. Albino wistar rats (180-200gms) of either gender will be retained in ordinary conditions of temperature (24±2°C), Rel. humidity (60±5%) and light(12 hr day & night cycles). They will be fed with usual pellet diet and water *as and when required.* All the experiments will be performed according to the norms of Ethical Committee.[10]

6) **Acute Oral Toxicity Survey:** Acute oral toxicity studies will be Conducted following OECD guidelines no. 423 by utilising the albino wistar rats. Dosage levels (100, 200, 500, 1000, 2000mg/kg) (Per Oral) will be well-thought-out to carry out acute oral toxicity study. The animals were selected to test the dose for observing the signs of toxicity & humanity for a period of 14 days.

7) **Experimental Design**

**Table 1: Experimental design.**

Albino wistar rats will be divided into eight groups of 6 animals in each.

| Grouping of animals | Age of the animals | Weight of the animals | Name of the drug | Dose & Route of administration |
|---|---|---|---|---|
| GROUP-1 | 12 Weeks | 150-200g | Normal saline (0.9%w/v) | 1ml/kg – p.o |
| GROUP-2 | 12 Weeks | 150-200g | Toxic – Gentamicin (GM) | 80mg/kg -i.p |
| GROUP-3 | 12 Weeks | 150-200g | GM + Standard drug (vit E + vit C) | 200+250mg/kg-p.o |
| GROUP-4 | 12 Weeks | 150-200g | GM + test drug1 | 200mg/kg -p.o |
| GROUP- 5 | 12 Weeks | 150-200g | GM + test drug1 | 400mg/kg-p.o |
| GROUP- 6 | 12 Weeks | 150-200g | GM + test drug2 | 200mg/kg-p.o |
| GROUP- 7 | 12 Weeks | 150-200g | GM + test drug2 | 400mg/kg-p.o |
| GROUP- 8 | 12 Weeks | 150-200g | GM + combination (test drug 1 + test drug 2) | 300+300 mg/kg-p.o |

**Experimental Method**: Albino wistar Rats will be apportioned into eight bunches of six animals each. Assemble 1 will be kept up as standard control. Assemble 2 to 8 will get gentamicin as harmful control. Assemble 3 will get the standard treatment. Assemble 4 and 5 will be treated with test sedate 1 and assemble 6 and 7 will be treated with test calm 2 in two unmistakable measurements exclusively. Accumulate 8 will be given a combination estimations of the two test drugs as the treatment. Gentamicin, standard sedate and both the extricates will be overseen for fifteen days inside the identical dosing arrange.

**Blood Sampler**: After dosing on 15th day, creatures will be kept for overnight fasting. On 16th day, blood tests will be collected by retro orbital cut utilizing capillary tubes to diagram parameters such as serum creatinine, BUN following to anti-oxidant markers such as MDA and glutathione levels.

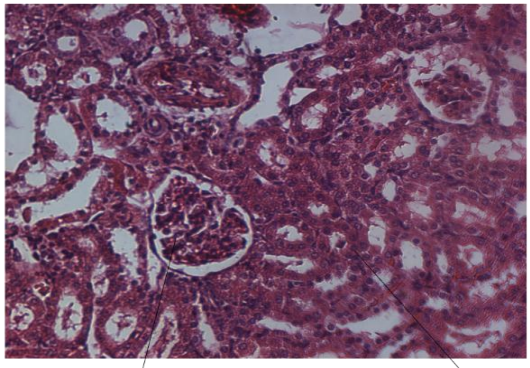
## RESULTS

**Table 2: Phytochemical Screening.**

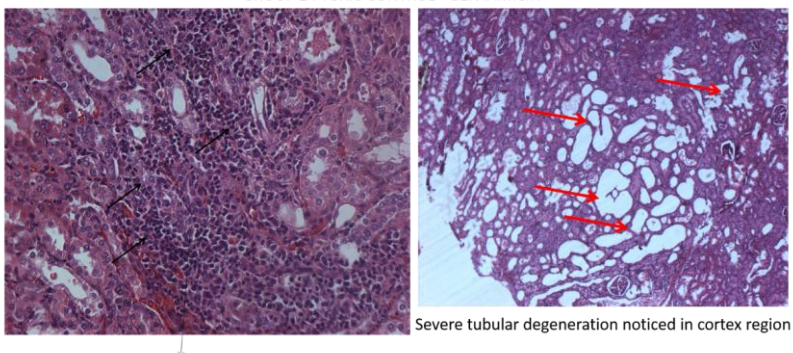
| Chemical constituent | Test | CA | OC |
|---|---|---|---|
| Tannins | Ferricchloride test | + | - |
| | Lead acetate test | + | - |
| Alkaloids | Mayer's test | - | + |
| | Dragoendroff's test | - | + |
| | Hager' test | - | + |
| | Wagner' test | - | + |
| Glycoside | | | |
| **A. Cardiac glycosides** | Legal's test | - | + |
| | Kellerkilliani test | - | - |
| | Liebermann's test | - | + |
| **B. Steroids** | Salkowskis test | - | + |
| | Liebermann's test | - | + |
| **C.Saponins** | Foam's test | + | + |
| **D. Flavonoids** | Schinoda's test | - | - |
| **E. Anthraquinones** | Borntragers test | - | - |
| | Modifiedborntrager's test | - | - |
| Carbohydrates | Molich test | + | + |
| | Fehlings test | - | + |
| | Benedicts test | - | + |
| Proteins | Biurets test | - | - |
| | Millons test | - | - |

## HISTOPATHOLOGICAL STUDIES

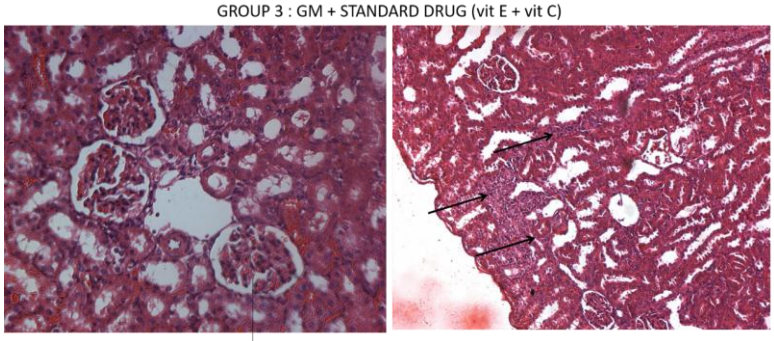
GROUP 1 : NORMAL CONTROL




GROUP 2 : TOXIC CONTROL - GENTAMICIN



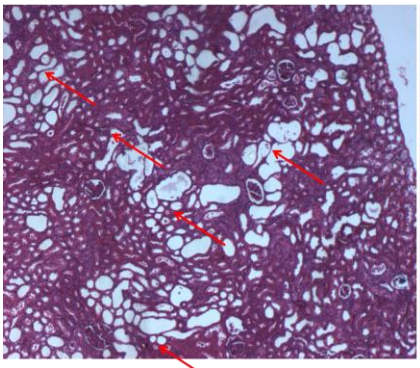
GROUP 3 : GM + STANDARD DRUG (vit E + vit C)



Glomerulus appeared normal

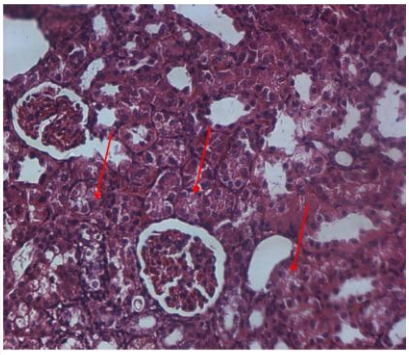
Multi focal regenerated tubules appeared in cortex region of kidney - arrow

GROUP 4 : GM + C.A. extract dose 1



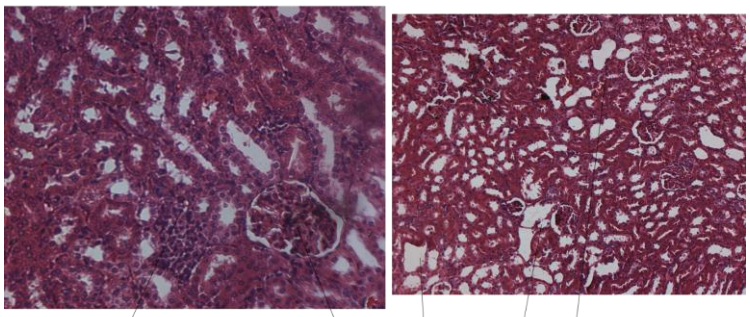
Moderate tubular dilatation and degeneration noticed in cortex region - arrow

GROUP 5 : GM + C.A. extract dose 2



Few regenerating tubules noticed in cortex region - arrow

GROUP 6 : GM + O.C. extract dose 1

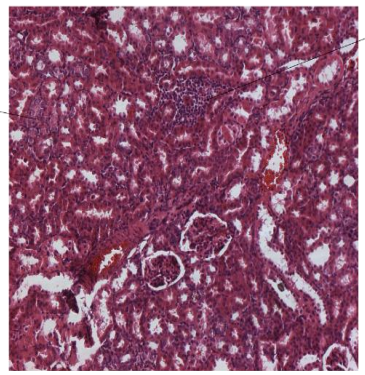


Tubular inflammation

Glomerulus appeared normal

Mild Tubular degeneration

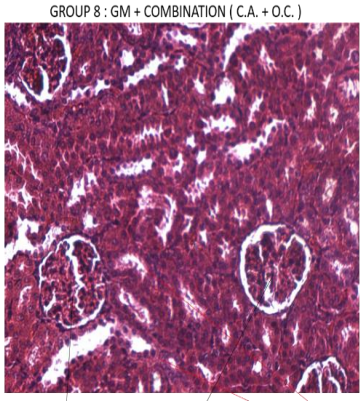
GROUP 7 : GM + O.C. extract dose 2



Few regenerating tubules

Foci of inflammation

GROUP 8 : GM + COMBINATION ( C.A. + O.C. )



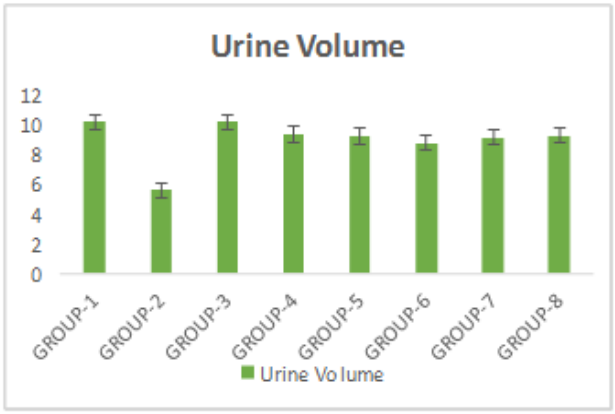
Normal glomerulus

Regenerating tubules

**ASSESSMENT OF BIOCHEMICAL PARAMETERS**

**Table 3: Assessment of urine volume.**

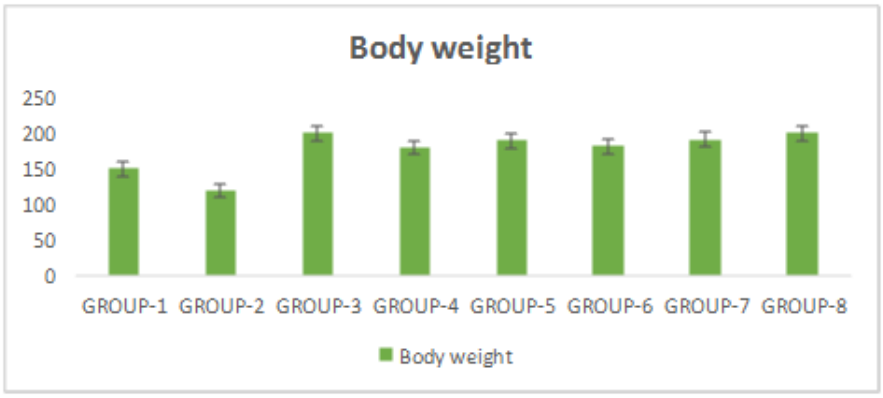
Group 3 has maximum urine volume




**Graph 1: Urine Volume.**

**Assessment of Body weight**

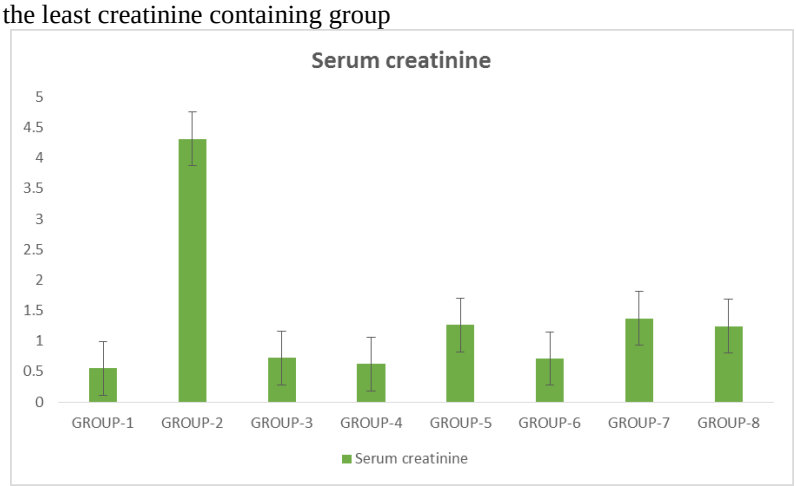
Group 3 and 8 have heaviest body weight of animals




**Graph 2: Body Weight.**

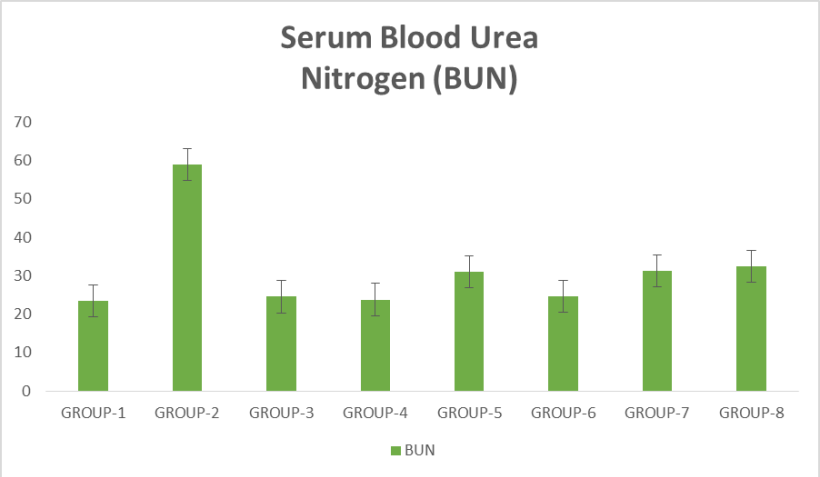
**Assessment of Serum creatinine level**

Group 1 & 3 has been the least creatinine containing group



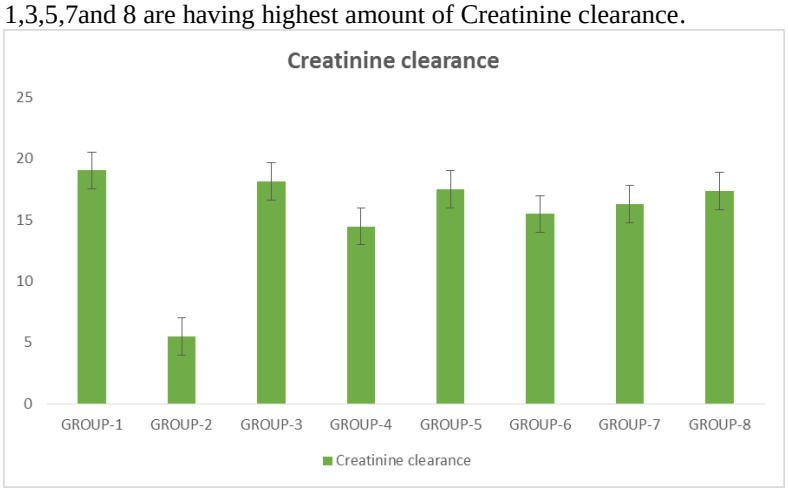

**Graph 3: Serum creatinine.**

**Table 6: Serum Blood urea nitrogen (BUN).**
Among extracts, Group 2 has the highest BUN level



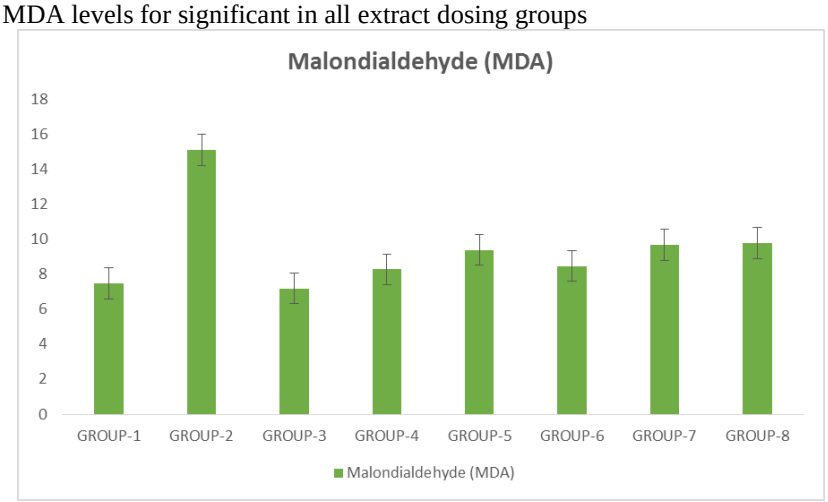

**Graph 4: Serum blood urea nitrogen (BUN).**

**Assessment of creatinine clearance**
Among extracts Group 1,3,5,7and 8 are having highest amount of Creatinine clearance.



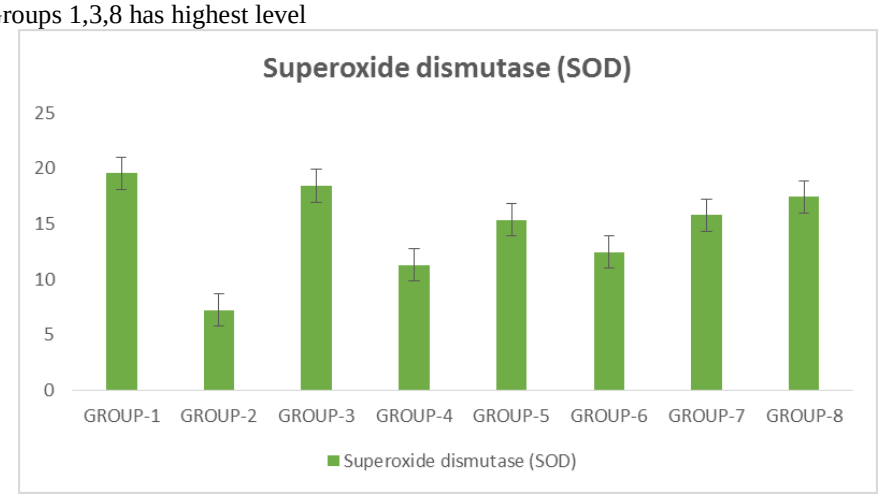

**Graph 5: Creatine Clearance.**

**Assessment of oxidative stress malondialdehyde (MDA) parameter**
Among extracts, the MDA levels for significant in all extract dosing groups



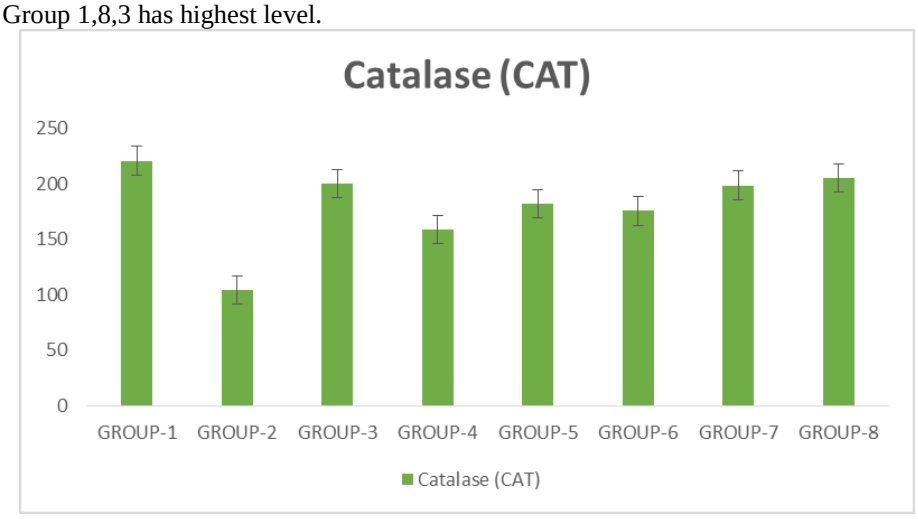

**Graph 6: MDA.**

**Assessment of superoxide dismutase (SOD)**
Among extracts Groups 1,3,8 has highest level



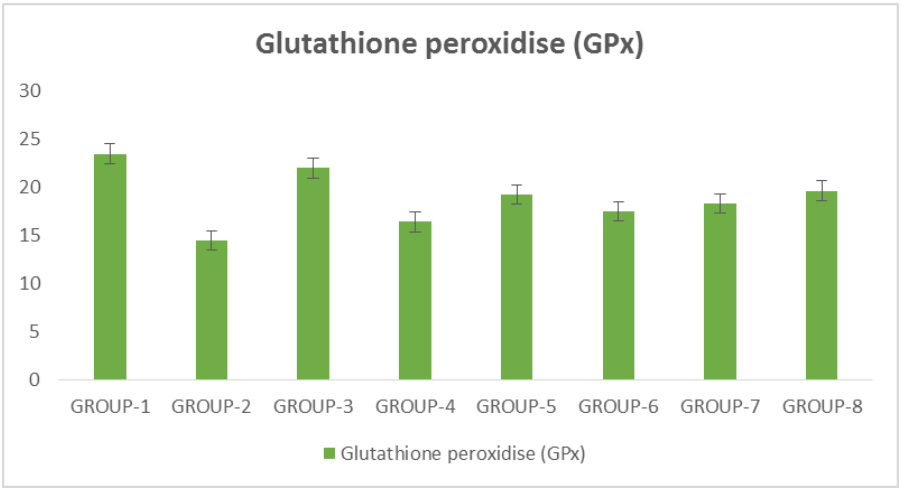

**Graph 7: SOD.**

**Assessment of Catalase (CAT)**
Among extracts Group 1,8,3 has highest level.




**Graph 8: CATAssessment of Glutathione peroxidise (GPx).**

Among extracts Group 1 & 3 has highest level




**Graph 9: Glutathione.**

## DISCUSSION

Renotoxicity is a pathological state characterized by a sudden and pronounced reduction in renal function, resulting in impaired clearance and abnormal retention of nitrogenous waste products such as serum creatinine and blood urea. This decline in renal performance leads to the accumulation of toxic metabolites that must normally be excreted to maintain homeostasis. The therapeutic landscape for acute kidney injury (AKI) remains limited, with only a few pharmacological agents available to restore renal function. Moreover, many synthetic renoprotective agents are associated with considerable side effects, which has driven a growing interest among researchers and the general public toward safer and more holistic approaches, particularly traditional and plant-based medicine.

For centuries, medicinal plants have been regarded as a cornerstone of renal care in traditional medical systems across the world. Before the advent of modern allopathic interventions, plant-derived preparations, often used in combination with dietary modifications, were the primary form of treatment for renal diseases. Ethnomedicinal plants continue to hold therapeutic significance as they offer potential benefits, such as reducing the risk of progression to end-stage renal failure, delaying the need for dialysis, and mitigating the adverse effects of long-term renal replacement therapy.

Phytochemical analysis of the ethanolic fruit pulp extract revealed the presence of a wide range of bioactive compounds, including flavonoids, terpenoids, alkaloids, tannins, saponins, and anthraquinones. These constituents, especially tannins, triterpenoids, flavonoids, and saponins, are well recognized for their potent antioxidant and cytoprotective properties. The acute oral toxicity studies performed according to OECD 423 guidelines (Acute Toxic Class Method) confirmed the safety of the ethanolic extract, as no lethality or toxic manifestations were observed even at higher doses, classifying the extract as non-toxic.

Gentamicin-induced nephrotoxicity is a widely accepted experimental model because it closely mimics human acute kidney injury. Gentamicin exerts nephrotoxic effects through multiple mechanisms: it damages both nuclear and mitochondrial DNA, promotes the excessive generation of reactive oxygen species (ROS), and activates apoptotic pathways, ultimately leading to necrosis of renal tubular cells. Additionally, gentamicin disrupts mitochondrial energy metabolism, further exacerbating renal damage. This model was therefore selected to scientifically evaluate the renoprotective efficacy of ethanolic extracts (EECA and EEOC).

## CONCLUSION

The results of the present study provide compelling evidence for the renoprotective potential of EECA and EEOC. Phytochemical screening confirmed the presence of several secondary metabolites—carbohydrates, alkaloids, flavonoids, glycosides, saponins, tannins, phenols, and anthraquinones—that may work synergistically to exert nephroprotective effects. Administration of gentamicin successfully induced oxidative stress, apoptosis, and necrosis, resembling human acute renal failure, validating the suitability of this model for nephrotoxicity research.

Treatment with EECA and EEOC produced multiple beneficial effects. There was a statistically significant improvement in physiological and biochemical parameters, including increased body weight, improved urine output, and enhanced creatinine clearance. Serum creatinine and blood urea nitrogen (BUN) levels, which were markedly elevated in gentamicin-treated animals, were significantly reduced after treatment. Furthermore, the extracts significantly decreased malondialdehyde (MDA) levels, a key marker of lipid peroxidation, thereby reducing oxidative damage.

Enzymatic antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), as well as non-enzymatic antioxidant reduced glutathione (GSH), were all significantly restored toward normal levels, suggesting that the extracts possess robust antioxidant activity. These findings are consistent with the known free-radical scavenging properties of flavonoids and phenolic compounds, which likely contributed to the observed renoprotection.

Histopathological examinations provided strong morphological evidence of the protective effects of EECA and EEOC. The extracts reversed gentamicin-induced renal tubular degeneration and necrosis, reestablished normal glomerular and tubular architecture, and restored overall kidney histology closer to the control group.

In conclusion, the ethanolic extract demonstrated significant renoprotective potential, attributable to its rich flavonoid and phenolic content and strong antioxidant activity. These findings highlight the therapeutic promise of EECA and EEOC as natural nephroprotective agents. However, further studies are warranted, including detailed histopathological investigations, bioassay-guided fractionation, and clinical evaluations, to isolate and characterize the active phytoconstituents and fully elucidate their mechanisms of action in renal protection.

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