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Evaluation of Gentamicin Induced Nephroprotector Activity of Hydroalcoholic Extract of Seeds of *Citrullus Lanatus*

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ABSTRACT

The present study was undertaken to evaluate the hydroalcoholic extract of seeds of *Citrullus lanatus* for its protective effect on gentamicin-induced nephrotoxicity in rats. The eight study groups contained six rats in each group. Nephrotoxicity was induced in male Wistar rats by subcutaneous administration of gentamicin 80 mg/kg.bd. wt. for nine days. Based on acute toxicity studies, doses of extract selected were 200 and 400mg/kg bd.wt. and administered orally for ten days. Effect of hydroalcoholic extract on gentamicin induced nephrotoxicity was determined using blood urea nitrogen, serum creatinine, serum total protein levels and urinary total proteins and urinary creatinine as indicators of kidney damage. Anti-oxidant studies were carried out and histological studies were also conducted in kidney tissue. Gentamicin induced nephrotoxicity which was indicated by increased levels of blood urea nitrogen, serum creatinine, serum total proteins, urinary total proteins and urinary creatinine levels. Extract alleviated the gentamicin-induced effects on serum markers and urinary functional parameters. Dose dependent decrease in LPO levels and increased levels of catalase, superoxide dismutase, glutathione also supported the nephroprotector activity of extract. In histopathological studies gentamicin induced nephrotoxicity was indicated by degenerative changes in kidney tissues, congestion with hemorrhages where as marked regenerative changes were observed in kidney sections of animals which received higher dose of the extract. Present study reveals that the hydroalcoholic extract of seeds of *Citrullus lanatus* attenuated the nephrotoxicity induced by Gentamicin in rats.

Keywords: *Citrullus lanatus*, Gentamicin, Nephroprotector activity, Anti-oxidants.

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INTRODUCTION

Drugs are being common source of acute kidney injury. Drug-induced Nephrotoxicity has become more common among certain patients and in specific clinical situations like chemotherapy, wide use of non steroidal anti-inflammatory drugs, Angiotensin converting enzyme inhibitors and Angiotensin II receptor blockers, anti-microbial agents, Vancomycin, etc[1]. Drugs such as Cisplatin, Gentamicin, Doxorubicin and toxic chemicals like cadmium, lead are some of the substances which result in a poisonous effect i.e., Nephrotoxicity. Gentamicin(GM) has been one of the commonest causes of drug induced Nephrotoxicity[2]. Because Gentamicin is a very effective antibiotic used in the treatment of several life threatening infections caused by various gram positive and gram negative aerobes, use of this drug is not discontinued [3]. Moreover it is still frequently used as a first- and second-choice drug in a vast variety of clinical situations because of its chemical stability, fast bactericidal effect, synergy with betalactamic antibiotics, little resistance, and low cost. It has been estimated that upto 30% of the patients who received treatment with Gentamicin for more than seven days show signs of renal impairment [4]. Gentamicin induces free radical production causing oxidative renal damage, possibly due to depletion of anti-oxidant systems[5]. Global estimates show that 80% of world population relies on traditional medicines derived from plant origin[6]. Ancient literature has prescribed various herbs for the treatment of renal disorders. *Citrullus lanatus* is one such plant seeds of which were used by folklore of Rayalaseema for their cooling property and diuretic effect[7-8]. Till date no systematic study has been carried out on seeds of *Citrullus lanatus* for nephroprotector activity. Therefore present work was planned to evaluate nephroprotective activity of hydroalcoholic extract of seeds of *Citrullus lanatus* (HAECL).

MATERIALS AND METHODS

Collection of Plant material:

Seeds of *Citrullus lanatus* used for this study were purchased from Srisu Agrichem Private Ltd., Kalyanpuri, Uppal and authenticated by Botanist Dr. Madhava Chetty, Herbarium keeper, Department of Botany, Sri Venkateswara University, Tirupati, India and specimen (Specimen Numbers: 640) has been deposited in Department of Botany, Sri Venkateswara University, Tirupati, India.

Preparation of hydroalcoholic extract:

Seeds were shade dried and powdered in Wiley mill. 200gm of seed powder was macerated with ethanol and water in the ratio of 60:40 for 24h and refluxed for 3 h, then filtered and subjected to

distillation under reduced pressure and the procedure was repeated for three times. Thus obtained hydroalcoholic extract of *Citrullus lanatus* (HAECL) was air dried and allowed to concentrate under reduced pressure to get the semi solid residue.

PHARMACOLOGICAL STUDIES

Pharmacological Evaluation:

The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals **(**CPCSEA**)**, India and ethical clearance for handling the animals was obtained from the Institutional Animal Ethical Committee **(**IAEC**)** prior to the beginning of the project work.

Animals:

Healthy adult male albino rats (150-200gm) of Wistar strain aged 60-90 days were used for this study. Rats were housed in polypropylene cages and maintained at standard conditions. The animals had free access to standard rat pellet food and tap water.

Acute toxicity studies[9]

Oral acute toxicity studies were conducted for the extract of seeds of *Citrullus lanatus* as per OECD guidelines no.423.

Assessment of Nephroprotector activity

Gentamicin was used for inducing nephrotoxicity in rats at a dose of 80mg/kg body weight (subcutaneously).

Treatment schedule for gentamicin induced nephrotoxicity

Group-I – Animals were administered with equivalent volumes of vehicle for nine days. (Normal control group)

Group-II. Animals received 80mg/kg/day s.c. of gentamicin for 9 days to induce nephritis and vehicle from day 10 to day 19. (Curative control Group)

Group-III. Animals received 80mg/kg/day s.c. of gentamicin for 9 days and Std. cystone from day 10 to day 19. (Standard group)

Group- IV - Animals received 80mg/kg/day s.c. of gentamicin for 9 days and lower dose (200 mg/kg body weight) of hydro alcoholic extract (p.o.) was given from day10 to day19. (Curative lower dose group)

Group- V - Animals received 80mg/kg/day s.c. of gentamicin for 9 days and higher dose (400 mg/kg body weight) of hydro alcoholic extract (p.o.) was given from day10 to day19. (Curative higher dose group)

Group-VI- Animals received vehicle from day 1 to day 10 and 80mg/kg/day s.c. of gentamicin from day 11 to day 19. (Prophylactic control group)

Group-VII- Animals received lower dose (200 mg/kg body weight) of hydro alcoholic extract from day 1 to day 10 and from day 11 to day 19 received 80mg/kg/day s.c. of gentamicin. (Prophylactic lower dose group)

Group-VIII- Animals received higher dose (400 mg/kg body weight) of hydro alcoholic extract from day 1 to day 10 and from day 11 to day 19 received 80mg/kg/day s.c. of gentamicin. (Prophylactic higher dose group)

Biochemical studies:

On day 19 urine was collected by keeping rats individually in metabolic cages; animals were sacrificed on day 20 by cervical decapitation and blood samples were collected through retro-orbital plexus. Nephroprotector potential of the extract was assessed by determination of serum markers' level and urinary functional parameters. Biochemical parameters such as Blood Urea Nitrogen (BUN) was estimated by Diacetyl mono-oxime method, Serum creatinine (SC) by Jaffe's Alkaline picrate method, Urinary total proteins by Turbidity method[10] and Creatinine clearance by Alkaline picrate method using commercial kits.

Cl_{cr} was calculated by using formula:

Creatinine clearance = Urinary creatinine / Serum creatinine X Urine volume/hr

Histopathological Studies:

Kidneys of two animals from each group were used for histological studies. The isolated kidneys were fixed in 10% buffered formalin and processed to paraffin wax. Sections (5 microns) were stained with haematoxylin and eosin and were examined under light microscope.

Anti-oxidant Studies:

Weighed portions of the kidneys were homogenized in ice cold 0.05 M phosphate buffer p^H 7.8 to obtain a 20% (w/v) homogenate. The homogenates were centrifuged at 10,000 rpm for 15 min and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes. Anti-oxidant studies were carried out by the estimation of levels of catalase (CAT) [11], superoxide dismutase(SOD), reduced glutathione (GSH) [12], and lipid peroxidation(LPO) [13] .

Statistical Analysis:

The statistical data was expressed as mean $\pm$ SEM. Parametric data which include all the biochemical parameters were analysed using one way Analysis of variance (ANOVA) and compared using Tukey-Kramer multiple comparison tests. A probability value of $p<0.05$ was considered as significant.

RESULTS AND DISCUSSION

Acute toxicity studies:

Animals which were observed for 14 days after single dosing of 2000mg/Kg. bd. wt. of hydroalcoholic extract of *Citrullus lanatus* showed neither any abnormal clinical signs nor mortality. So the extract was found to be safe at 2000mg/kg. bd. wt. Depending on acute toxicity studies nephroprotector activity of extract was tested at two different dose levels i. e., 200 mg/kg body wt. and 400 mg/kg body wt. To assess the nephroprotector activity of the extract, the data obtained for the extract treated groups (Groups IV and V) was compared with that of Group II (Curative control) animals which received only Gentamicin from day 1 to day 9. In the same way the data of Group VII and VIII animals was compared with that of Group VI (Prophylactic control) which received only Gentamicin from day 11 to day 19.

Effect of hydroalcoholic extract of seeds of *Citrullus lanatus* against gentamicin induced nephrotoxicity:

Nephrotoxicity is one of the most important side effects and therapeutic limitations of Gentamicin14. Gentamicin mainly gets accumulated in epithelial tubular cells causing tubular necrosis, activation of apoptosis and massive proteolysis. It also causes cell death by generation of free radicals, phospholipidosis, extracellular calcium-sensing receptor stimulation and energetic catastrophe, reduced renal blood flow and inflammation4. Though chemical research was aimed at obtaining intrinsically less toxic compounds only modest success was achieved. Day by day the number of people suffering from kidney disorders is increasing at an alarming rate but no drug in allopathy is able to combat this renal toxicity efficiently. This dire need for nephroprotector agents results in exploitation of medicinal plants. Previously it was reported that plants like Solanum xanthocarpum, Aerva lanata, Crataeva nurvula, Strychnos potatorum, Orthosiphon stamineus, Bauhinia variegata, Emblica officinalis, Tribulus sativus, Cassia auriculata etc. possess significant nephroprotector activity against Gentamicin induced nephrotoxicity15-17. So the present study was aimed at investigation of nephroprotector activity of seeds of Citrullus lanatus. Urea is the chief excretory product of protein metabolism. It is formed in the liver mainly by the breakdown of amino acids. Blood urea nitrogen levels may be raised in various pathological conditions primarily in renal diseases like acute and chronic nephritis, acute tubular nephrosis, hepato-renal syndrome, uraemia due to fall in glomerular filtration rate18. Creatinine represents the waste products of creatine metabolism and it arises in the body from the spontaneous breakdown of creatine phosphate. It is considered to be a reliable indicator of kidney function. It is normally filtered by

the glomeruli. As its excretion is not related to the food protein its variation in the excretion indicates the metabolic disorders18. Elevated creatinine level signifies impaired kidney function or kidney disease. Serum total proteins also known as total protein mainly constitute the mixture of plasma albumin and globulin. General causes of alterations of serum total protein are a change in the volume of plasma water and a change in the concentration of one or more of the specific proteins in the plasma. In case of renal diseases high globulin levels and low albumin levels may be observed19-20. Earlier reports suggested that administration of GM resulted in renal injury as a consequence of tubular necrosis21. Recent reports evidenced that seeds of *Elaeocarpus ganitrus*, garlic extract, decoction of root bark of Berberis aristata, Ferula foetida prevented the increase in the levels of serum creatinine, BUN, total proteins in blood against GM-induced nephrotoxicity21-24. The mechanism of this protective effect might be due to anti-oxidant potential of the extracts obtained from medicinal plants. In the present investigation, our results coincided with earlier reports i.e., treatment with higher dose of HAECL extract i.e., 400mg/kg body weight significantly reduced the levels of serum and urinary parameters which may be due to the prevention of tubular necrosis and glomeruli damage. Table:1 shows that single daily subcutaneous administration of 80 mg/kg body weight of Gentamicin for 9 days caused significant raise in the levels of serum creatinine, total proteins and blood urea nitrogen in the Gentamicin treated rats (Group- II and Group VI) when compared with Group-I. However, elevation in the levels of these serum parameters were significantly attenuated by treatment with the hydroalcoholic extract (HAECL) of seeds of Citrullus lanatus (Group IV, V and Group VII and VIII) in a dose related manner. Plasma proteins are not normally filtered through the glomeruli, but in kidney diseases, due to the alteration in glomerular permeability, these appear in urine. Animals which received gentamicin alone exhibited high amount of protein excretion in urine, which indicates that it induced renal damage significantly where as animals treated with higher dose of HAECL (groups V and VIII) reverted the effect caused by gentamicin. Table: 2 shows that animals which received Gentamicin alone (Group II and Group VI) exhibited decreased levels of Creatinine clearance when compared with normal control group animals. On Oral administration of HAECL 200mg/kg body weight in Groups IV and VII and 400mg/kg body weight in Groups V and VIII respectively, showed significant increase in Creatinine clearance. Animals which received Gentamicin alone (Group II and Group VI) excreted high amount of Urinary proteins when compared to normal control animals. Administration of HAECL with doses of 200mg/kg and 400 mg/kg to Group IV, V (lower and higher dose treated curative groups) and Group VII and VIII (lower and higher dose treated

Prophylactic groups) animals reversed the effect caused by gentamicin by exhibiting dose related protection. Free radical generation plays a crucial role in the pathogenesis of renal damage associated with the use of gentamicin. Moreover, the oxidative damage also has been supported by the fact that the administration of several compounds with anti-oxidant properties, ROS scavengers, and/or anti-oxidant enzymes are able to ameliorate the severity of GM-induced renal damage. SOD is the first line of defense against free radical induced oxidative stress and responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radical to hydrogen peroxide. GSH is an intracellular reductant and plays an important role in catalysis, metabolism, and transport. It protects cells against peroxides, free radicals, and other toxic compounds25. CAT is a key component of the anti-oxidant defence system and catalyses the reaction of hydrogen peroxide into water26. Gentamicin caused significant decrement in the levels of GSH, SOD and catalase in kidney, indicating oxidative stress25. In the present investigation HAECL ameliorated the GM-induced oxidative stress which implicates its anti-oxidant and nephroprotective activity. LPO is an autocatalytic process, which is a common reason for cell death. Malondialdehyde (MDA) is one of the end products in the LPO procedure. It is generated during oxidative degeneration as a product of free oxygen radicals, which is accepted as an indicator of LPO. In the present investigation,

administration of gentamicin caused increase in renal concentration of MDA. This indicated increased levels of LPO which showed the renal oxidative stress The elevated level of LPO was decreased by the administration of HAECL (400 mg/kg) which depicts its potent anti-oxidant activity. Table 3 represents the effect of anti-oxidant enzyme levels on gentamicin induced (Group II and Group VI) rats alone and on extract treated rats (Group IV, V and VII, VIII). The levels of anti-oxidants such as catalase, SOD and glutathione declined significantly than that of normal control animals (Group-I) where as lipid peroxidation levels were increased in Group II and Group VI rats. Co-administration of hydroalcoholic extract of *Citrullus lanatus* at the doses of 200 and 400 mg/kg for 10 days (Group IV, V and to Group VII, VIII) markedly prevented these Gentamicin induced alterations and maintained enzymes level nearer to normal values. Standard (cystone) treated animals (Group-III) also significantly increased the levels of glutathione, SOD and Catalase and decreased the levels of lipid peroxidation in Gentamicin induced toxic rats. HAECL at the dose of 400 mg/ kg, p.o., had improved the glutathione, SOD and catalase levels significantly, which were comparable with standard, Cystone. In histopathological studies the sections of kidney of normal control rats showed normal architecture of renal tubules and renal corpuscles. The Bowman's capsule and the glomeruli appeared to be prominent and normal. In the

gentamicin control group, the kidney showed congestion with haemorrhages, vacuolization, necrotic changes, dilated proximal convoluted tubules, slogging of epithelium due to desquamation and atrophic glomeruli which were signs of gentamicin induced nephrotoxicity. Sections of the kidney of the rats treated with gentamicin and HAECL (400 mg/kg) showed marked improvement in comparison with gentamicin control group, and it reverted the histological appearance seen in the induced group. Histopathological studies on rat kidney tissues were represented from figure 1 to 8 which revealed that kidney histological sections of rats treated with gentamicin and higher dose of HAECL (400 mg/kg) of curative group (Group-V) and prophylactic groups (Group-VIII) showed marked improvement showing normal kidney histology and architecture.

Table: 1 Effect of hydroalcoholic extract of seeds of *Citrullus lanatus* against Gentamicin induced nephrotoxicity on serum parameters

Group	Treatment	BUN (mg/dl)	SC (mg/dl)	S_{TP} (g/dl)
I	Normal (vehicle)	16.82±0.22	0.73±0.03	5.91±0.13
II	Curative control Gentamicin (80mg/kg bd. wt)	30.73±0.75[a]	1.55±0.10[a]	8.12±0.14[a]
III	Standard (cystone 5ml/kg. bd. wt.)	17.5±0.43	0.78±0.05	6.0±0.09
IV	Gentamicin (80mg/kg bd. wt) for 9 days + Lower dose of the extract (HAECL) from day 10 to 19.	24.77±0.66[b]	1.08±0.04[d]	7.35±0.11[e]
V	Gentamicin (80mg/kg bd. wt) for 9 days + higher dose of the extract from day 10 to 19.	16.853±0.18[b]	0.87±0.03[b]	6.68±0.15[b]
VI	Prophylactic control Gentamicin from day 11 to day 19.	38.18±0.73[a]	1.62±0.10[a]	8.39±0.13[a]
VII	Lower dose of the extract from day 1 to day 10 + Gentamicin (80 mg/kg bd. Wt.) from day 11 to 19.	30.71±0.4[c]	1.18±0.05[d]	7.15±0.12[c]
VIII	Higher dose of the extract from day 1 to day 10 + Gentamicin (80 mg/kg bd. Wt.) from day 11 to 19.	23.57±0.52[c]	0.97±0.03[c]	6.75±0.15[c]

Each value represents the Mean ± S.E.M from 6 animals in each group. a: $p<0.0001$ when compared with normal group. b: $p<0.0001$ when compared with curative control c: $p<0.0001$ when compared with prophylactic control d: $p<0.001$ when compared with prophylactic control e: $p<0.01$ when compared with curative control.

Table: 2 Effect of hydroalcoholic extracts of seeds of *Citrullus lanatus* against gentamicin induced nephrotoxicity on urinary parameters

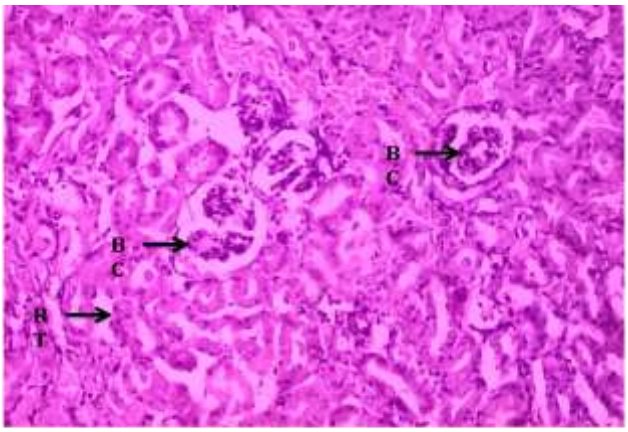
Group	Treatment	Cl_{cr} (ml/hr/100g bd.wt)	U_{TP} (mg/24hrs)
I	Normal (vehicle)	16.48±0.58	7.07±0.13
II	Curative control Gentamicin (80mg/kg bd. wt) from day 1 to day 9.	6.25±0.21[a]	17.22±0.24[a]
III	Standard (cystone 5ml/kg. bd. wt.)	15.38±0.28	7.58±0.17
IV	Gentamicin (80mg/kg bd. wt) for 9 days + Lower dose of the Extract (HAECL) from day 10 to 19.	8.92±0.22[b]	12.38±0.2[b]
V	Gentamicin (80mg/kg bd. wt) for 9 days + higher dose of the extract (HAECL) from day 10 to 19.	13.3±0.24[b]	7.72±0.18[b]
VI	Prophylactic control Gentamicin from day 11 to day 19.	5.68±0.19[a]	26.2±0.27[a]
VII	Lower dose of the extract (HAECL) from day 1 to day 10 + Gentamicin (80 mg/kg bd. Wt.) from day 11 to 19.	8.13±0.22[c]	18.28±0.21[c]
VIII	Higher dose of the extract (HAECL) from day 1 to day 10 + Gentamicin (80 mg/kg bd. Wt.) from day 11 to 19.	10.32±0.17[c]	11.87±0.15[c]

Each value represents the Mean ± S.E.M from 6 animals in each group. a: $p<0.0001$ when compared with normal group. b: $p<0.0001$ when compared with curative control c: $p<0.0001$ when compared with prophylactic control.

Table: 3 Effect of hydroalcoholic extract of seeds of *Citrullus lanatus* on anti-oxidant levels in gentamicin induced nephrotoxic rats

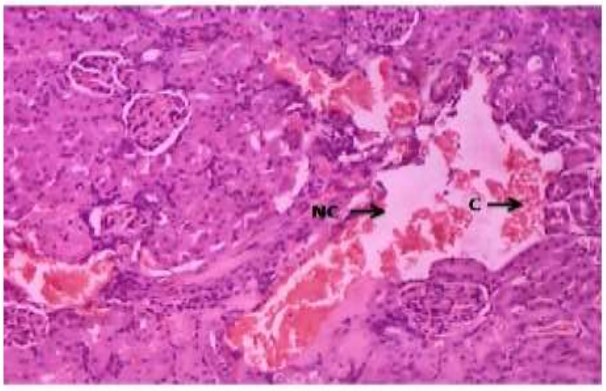
Group	LPO nm/100mg of tissue	SOD units/mg of tissue	CAT units/mg of tissue	GSH nm/100mg of tissue
I Normal	0.97±0.13	12.8±0.27	43.88±0.68	20.08±0.45
II Curative control	4.67±0.46[a]	6.27±0.21[a]	32.92±0.95[a]	11.72±0.21[a]
III Standard	1.32±0.13	12.27±0.56	63.13±0.52	18.93±0.21
IV Curative lower dose	2.58±0.20[b]	8.53±0.21[b]	34.4±0.60[b]	15.45±0.21[b]
V Curative higher dose	1.27±0.17[b]	12.1±0.17[b]	46.6±0.84[b]	18.75±0.35[b]
VI Prophylactic control	5.42±0.29[a]	5.95±0.16[a]	16.08±0.87[a]	10.92±0.18[a]
VII Prophylactic lower dose	3.1±0.25[c]	7.82±0.21[d]	22.97±0.95[c]	13.87±0.25[c]
VIII Prophylactic higher dose	1.85±0.26[c]	10.38±0.23[c]	39.32±0.94[c]	16.8±0.43[c]

Each value represents the Mean ± S.E.M from 6 animals in each group. a: p<0.0001 when compared with normal group. b: p<0.0001 when compared with curative control, c: p<0.0001 when compared with prophylactic control, d: p<0.001 when compared with prophylactic control.



Group-I Kidney (Body) 10X

Figure 1: Section of rat kidney showing normal organization of glomeruli



GROUP II KIDNEY 10X

Figure 2: Section of rat showing necrotic changes in kidney tissues and congestion with haemorrhages (Curative control)

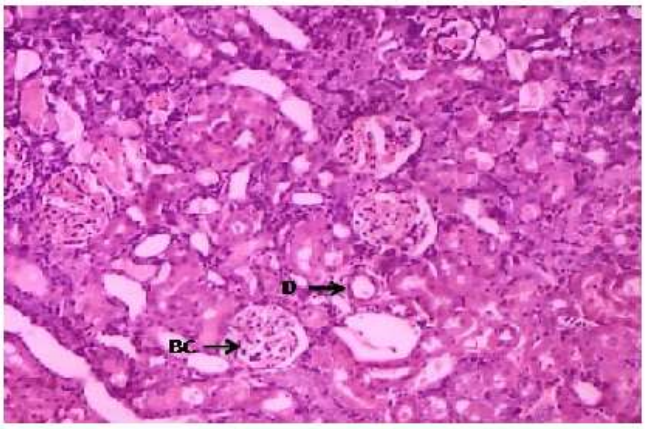


Figure 3: Section of rat kidney treated with standard (cystone) showing almost normal organization of Bowman's capsule and distal convoluted tubule

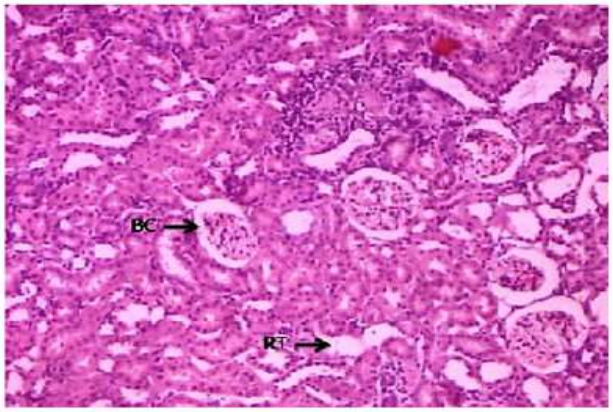


Figure 4: Section of rat kidney showing mild regenerative changes in kidney tissue (curative lower dose)

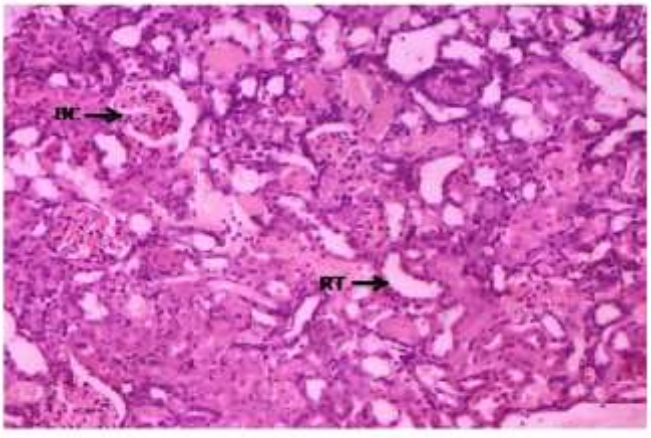
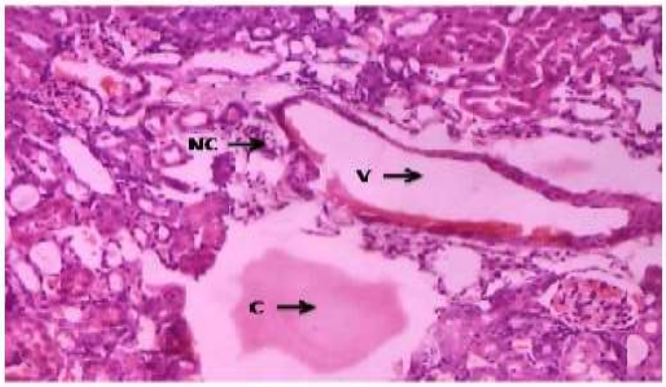
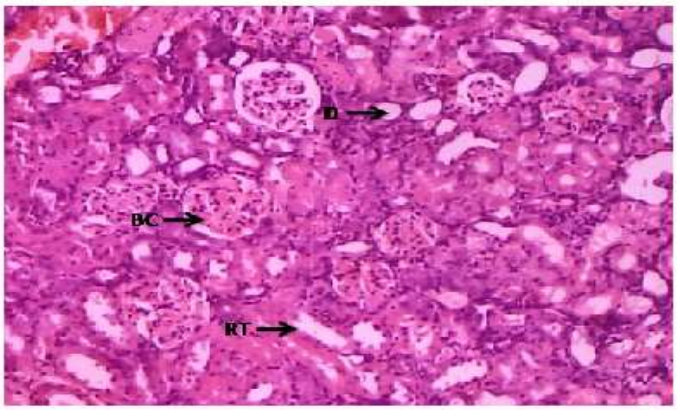


Figure 5: Section of rat kidney showing marked regenerative changes in RT and BC (curative higher dose)



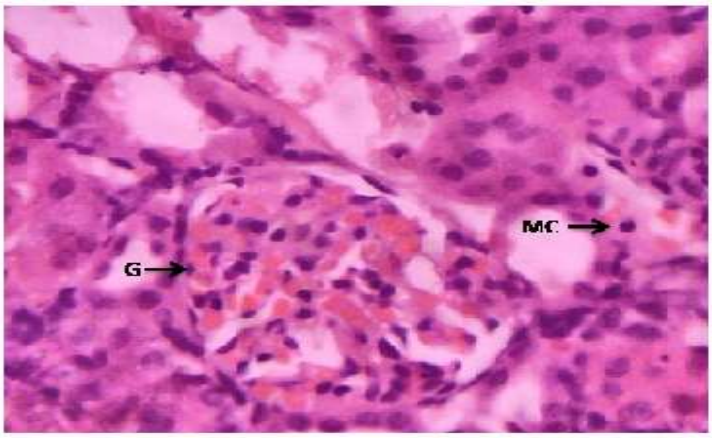
GROUP VI KIDNEY 10X

Figure 6: Section of rat kidney showing vacuolization, congestion and necrotic changes (Prophylactic control).



GROUP VII KIDNEY 10X

Figure 7: Section of rat kidney showing mild regeneration of kidney tissue (prophylactic lower dose)



GROUP VIII KIDNEY 40 X

Figure 8: Section of rat kidney showing regenerative changes in kidney tissue and similar to normal cyto-architecture (prophylactic higher dose)

CONCLUSION

Based on this study it can be concluded that hydroalcoholic extract of *Citrullus lanatus* possesses significant nephroprotector activity, this may be due to its anti-oxidant properties. *Citrullus lanatus* may be employed in protecting renal tissue from drug induced pathogenesis and oxidative stress.

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REFERENCES

1. George SP, Anushree CS. Drug-induced impairment of renal function. Int J Nephrol Renovasc Dis. 2014; 7: 457–68.
2. Walker RJ, Duggin GG. Drug nephrotoxicity. Annu. Rev. Pharmacol. Toxicol. 1988; 28: 331-45.
3. Choi JJ, Moffett BS, McDade EJ, Palazzi DL. Altered gentamicin serum concentrations in obese pediatric patients. Pediatr Infect Dis J 2011; 30: 347–9.
4. Ali BH, Mohammed Al Zabi, Experimental gentamicin nephrotoxicity and agents that modify it: A mini-review of recent research. Basic Clin Pharmacol Toxicol, 2011; 109(4): 225-32.
5. Azab ESA, Fathy AF,Albasha MO. Nephro-protective effects of curcumin, rosemary and propolis against gentamicin induced toxicity in guinea pigs: Morphological and biochemical study. Am J Clinical and Experimental Medicine 2014; 2(2): 28-35.
6. Martins E. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in Pharmacology. 2013; 4:177.Published online 2014 Jan 10.
7. Kirtikar KR, Basu BD. Indian medicinal plants. International Book Distributor, Dehradun, India, 2005, pg. 1149.
8. Madhava chetti, *et al.*, Flowering Plants of Chittoor District Andhra Pradesh, India. 2008; pg 137.
9. Oral acute toxicity studies, OECD guidelines no. 423.
10. Godkar PB. “Kidney function tests in text book of medicinal laboratory”, Bhalani publishing house, Bombay 1994.

11. Cohen, D., Dembiec, D., Marcus, J., 1970. Measurement of catalase activity in tissue extracts. Annals Biochemistry. 1970; 34: 30–38.
12. Ellman GL. 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82: 70-7.
13. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-58.
14. Jose M lopez novoa New insights into the mechanism of aglyco nephrotoxicity. Kidney Int 2011; 79(1): 33-45.
15. Talib H, Ramesh KG, Sweety K, Bavani E, Vijayakumar M, Chandana Venkateswara Rao, Nephroprotective activity of *Solanum xanthocarpum* fruit extract against gentamicin–induced nephrotoxicity and renal dysfunction in experimental rodents. Asian Pacific J Tropical Medicine. 2012; 5(9): 686–91.
16. Mohana lakshmi. S, Usha kiran reddy. T and Sandhya rani. K "A review on medicinal plants for nephroprotective activity" Asian J Pharm Clin Res 2012; 5(4): 8-14.
17. Kanchan Gaikwad, Pradeep Dagle, Pushpalata Choughule, Y. M. Joshi, Vilasrao Kadam A review on some nephroprotective medicinal plants. Int J Pharma Sci Res 2012; 3(8): 2451-54.
18. S. R. Kale, R. R. Kale. Practical Biochemistry and clinical pathology. (as per E. R. -1991) 4th edition September 1995 pg no. 45,47.
19. Chernecky CC, Berger BJ (2013). Laboratory Tests and Diagnostic Procedures, 6th ed. St. Louis: Saunders.
20. Pagana KD, Pagana TJ (2010). Mosby's Manual of Diagnostic and Laboratory Tests, 4th ed. St. Louis: Mosby.
21. Rahul Motiram Kakalij, Chaitanya P. Alla, Rahul P. Kshirsagar, Boyina Hemanth Kumar, Sumeet S. Mutha, andPrakash Vamanrao Diwan Ameliorative effect of *Elaeocarpus ganitrus* on gentamicin-induced nephrotoxicity in rats. Indian J Pharmacol. 2014; 46(3): 298–302.
22. Pedraza CJ, Maldonado PD, Medina Campos ON, Olivares-Corichi IM, Granados-Silvestre MA. Garlic ameliorates gentamicin nephrotoxicity: relation to antioxidant enzymes. Free Radic Biol Med. 2000; 29(7):602-11.
23. Sreedevi adikay, Bharathi koganti, Prasad KVSRG, effect of decoction of root bark of *Berberis aristata* against cisplatin induced nephrotoxicity in rats. Int J Pharm Pharma Sci 2010; 2(3): 51-6.

24. Rasikh J, Mohd A, Raheela J, Qudsia N, Kalim J. Extract of *Ferula foetida* regel reverses gentamicin induced nephrotoxicity in rats. EXCLI J. 2012; 11:760-66.
25. Rajneesh Kumar Singh, Rupesh KG, Karchuli MS. Evaluation of nephroprotective activity of *Mentha arvensis* in cisplatin-induced nephrotoxicity. Asian J Pharm Clin Res, 2014; 7(4), 188-91.
26. Suji Arivazhagan JJ, Vimalastalin R. Nephroprotective activity of A*ristolochia indica* leaf extract against gentamicin induced renal dysfunction. Int J Res Biochem Biophysics 2014; 4(2): 13-8.