M.Aparna rama laxmi devi et al /J. Pharm. Sci. & Res. Vol. 8(9), 2016, 995-1007

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Evaluation of Nephroprotective Activity of Ethanolic Extract of *Annona reticulata* in Gentamicin and Cisplatin Induced Nephrotoxicity in Rats

M.Aparna rama laxmi devi[1], M.Yaso Deepika[2], B.Nagaraju[3] , K.Prasad[4].

[1] *Department of Pharmacology, Shri Vishnu College of Pharmacy,Vishnupur, Bhimavaram,West Godavari,A.P.India.*

[2] *Department of Pharmacology, Anurag group of institutions,Ghatkesar, Hyderabad.*

[3]*Department of Pharmacology, Chebrolu Hanumaiah institute of Pharmaceutical Sciences, Chowdavaram, Guntur, AP, India.*

[4] *Department of Pharmacology, Shri Vishnu College of Pharmacy, Vishnupur,Bhimavaram,West Godavari, A.P.India*

Abstract

Objective: The Aim of the study was to investigate the nephroprotective activity of ethanolic extract Of *Annona reticulata* in gentamicin and cisplatin induced nephrotoxicity in rats.

Methods: Ethanolic extract of aerial parts of *Annona reticulata* plant was studied for its Nephro-protective activity in animal experimental models.Nephrotoxicity was induced by Gentamycin and cisplatin Nephro-protective activity of ethanolic extract of *Annona reticulata* plant aerial parts at doses 250 mg/kg p.o. and 500 mg/kg p.o. in models of nephro-protective activity viz. gentamicin and cisplatin induced nephrotoxicity.The study period is 24 days and 15 days for gentamicin and cisplatin induced nephrotoxicity models.

Results: In gentamicin treated groups (2^{nd} and 5^{th}) of animals the concentration of serum, urea, Creatinine, Uric acid, Total protein and Urine Urea, uric acid, creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. In Cisplatin treated groups (2^{nd} and 5^{th}) of animals the concentration of serum urea, Creatinine, Uric acid, Total protein and Urine Urea, uric acid, creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity.

Conclusion: On evaluating biochemical parameters it was found that the ethanolic extract of aerial parts of *Annona reticulata* showed nephro-protective activity in both Gentamycin and cisplatin induced nephrotoxicity the models due to presence of therapeutic phyto constituents.

Key words: *Annona reticulata*, Gentamicin, Cisplatin, Nephro-Protective activity

INTRODUCTION

Medicinal plants have been identified and used throughout human history. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals. At least 12,000 such compounds have been isolated so far; a number estimated to be less than 10% of the total. [1, 2]. Various drugs available in the market for nephro-protective activity and none of the drug is not up to mark for showing its efficacy. From literature survey it was found that *Annona reticulata* effective in treatment of boils, abscesses and ulcers and used in dysentery and darrhoea, daibetes, insecticide, antiovulatory and abortifacient, hair tonic, malaria and syphilis [3-6]. The study period is 24 days and 15 days for gentamicin and cisplatin induced nephrotoxicity models. Animals used are male wistar rats in both models. Before performing the Nephro-protective activity of ethanolic extract of the plant aerial parts, phytochemical evaluation was done. Nephrotoxicity has been related to a selective accumulation of gentamicin in the renal cortex. Morphologic lesions of proximal tubules have been documented in optic microscopy. At the ultra-structural level, the earliest lesions observed concern lysosomes, which show an accumulation of myeloid bodies due to genearation of free oxide anions [7-15]. The cysteinyl-glycine-conjugates of Cisplatin are further metabolized to cysteine-conjugates causes the renal damage[16-18].

MATERIALS AND METHODS

Material collection

The plant was collected during the month of March 2014, from Tekisettipalem and Anthervedi palem and Gudemellanka villages.The plant was authenticated by Head of the Department of Botany, D.N.R. College of pharmacy, Bhimavaram. Aerial parts of *Annona reticulata* were dried at room temperature for 2-3 days. The dried aerial parts of *Annona reticulata* was powdered . The extraction was done by using the process of soxhlet extraction. 150 grams of fine powder was suspended in 400 mL ethanol for 48 hours at 65 degrees of temperature soxhlet extractor. After 48 hours the extract was taken and residue was dried[19].

Experimental Animals

Male wistar rats of 150-200 grams weighed were used for present study. The animals were housed in polypropylene cage (6 animals per cage), the standard conditions were maintained (12 hours light and 12 hours dark cycle, 23 ± 5° C and 40-60% humidity). The standard rat diet, water was provided ad libitum. All the animals were collected from the central animal house SICRA Labs Pvt Ltd, IDA-Kukatpally, Hyderabad and all experiments were conducted according to the ethical norms approved by CPCSEA, Ethical Committee IAEC reg.no. 769/2011/CPCSEA).

Preliminary Phytochemical Screening

The ethanolic extract of aerial parts of *Annona reticulata* were found chemical constituents like Alkaloids, glycosides, saponins, flavonoids, Fixed oils and fats,

Phytosterols and tannins after the performing of qualitative phytochemical analysis[20-25].

Experimental Procedure

Induction of Nephrotoxicity

Induction of Nephrotoxicity By Gentamicin [26].

The Nephrotoxicity in this model was induced by 80 mg/kg weight of animal by intra muscular route administration. The study period is 14 days and 24 days in preventive and curative regimen respectively.

Induction of Nephrotoxicity By Cisplatin [27].

The obesity induced by injection of Cisplatin through single intra-peritoneal administration. The dose required for induction is 5 mg/kg.

Preparation of Test Drug

The test drugs were prepared by 2% tween 80. Both standard and test drugs were given by oral gavage i.e. per oral route at a dose of 0.4 ml/kg body weight. All drugs were prepared freshly before administration.

EXPERIMENTAL PROCEDURE

Induction of Nephrotoxicity by Gentamicin [27].

Seven groups of six rats each were used for the study.

Group I: Animals were orally administered with gum acacia solution (2%w/v) for 23 days.

Group II: animals treated with only gentamicin for 13 days, blood was withdrawn on the 14th day.

Group III: Administered with gentamicin for 13 days (40mg/kg body wt., s.c.) and treated with EEAR 250mg/kg per oral for 13 days

Group IV: Administered with gentamicin for 13 days (40mg/kg body wt., s.c.) and treated with EEAR 500 mg/kg per oral for 13 days

Group V: Administered with gentamicin for 23 days (40mg/kg body wt., s.c.) only

Group VI: Administered with gentamicin for 13 days (40mg/kg body wt., s.c.) and treated with EEAR 250mg/kg per oral from 14th day onwards till 24th day

Group VII: Administered with gentamicin for 13 days (40mg/kg body wt., s.c.) and treated with EEAR 500mg/kg per oral from 14th day onwards till 24th day

The 2nd and 3rd groups are studied for preventive regimen whereas 6th and 7th groups are studied for curative regimen. Blood was withdrawn from the rat by retro orbital puncture method and animals were sacrificed for isolation of organs on 14th day and 24th day from preventive and curative regimen animals respectively

Induction of Nephrotoxicity by Cisplatin [28]

Group I: Animals were orally administered with gum acacia solution (2%w/v) for 15 days.

Group II: Treated with single administration of cisplatin (5 mg/kg i.p.); blood was withdrawn on 6th Day.

Group III: Treated with single administration of cisplatin (5 mg/kg i.p.); and treated with EEEAR 250mg/kg per oral for 6 days

Group IV: Treated with single administration of cisplatin (5 mg/kg i.p.); and treated with EEAR 500 mg/kg per oral for 6 days

Group V: Treated with single administration of cisplatin (5 mg/kg i.p.); and blood was withdrawn on 15th day

Group VI: Treated with single administration of cisplatin (5 mg/kg i.p) and treated with EEAR 250 mg/kg per oral from 6th day onwards till 15th day

Group VII: Treated with single administration of cisplatin (5 mg/kg i.p.); and treated with EEAR 500 mg/kg per oral from 6th day onwards till 15th day

The 2nd and 3rd groups are studied for preventive regimen whereas 6th and 7th groups are studied for curative regimen. Blood was withdrawn from the rat by retro orbital puncture method and animals were sacrificed for isolation of organs on 6th day and 15th day from preventive and curative regimen animals respectively.

At the end of experimental period, all the animals were sacrificed under diethyl ether anaesthesia. Blood samples were collected from the rat by using retro orbital puncture method , allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters

Biochemical parameters

On respective day of completion of studies, blood was collected from rats by retro orbital puncture method and subjected to Biochemical parameters i.e., Estimation of Blood urea, Creatinine, Uric acid, Total protein were analyzed estimations by using prietest biochemical kits by ROBONIK biochemical analyzer. Haematological parameters like RBC and WBC are estimated to know the kidney function [29].

Histopathology of Kidney

The kidney was removed, weighed and morphological changes were observed. A 10% of kidney homogenate was used for antioxidant studies such as, superoxide dismutase (SOD).

Statistical Analysis

The obtained results were expressed as Mean ± SEM. Comparison between control and treatment groups were performed by one way analysis of variance (ANOVA) followed by Dunnet's test. The statistical significance criterion was $p< 0.05$ (95% level). $P<0.05$ is considered as significant.

RESULTS

Blood urea, Creatinine, Uric acid, Total protein, RBC, WBC estimations were performed and treatment groups are compared with disease control i.e. disease control groups. And the statistical analysis was done by one way analysis of variance (ANOVA) followed by Dunnets test and results were found significant.

In Gentamicin Induced Model

In gentamicin treated groups (2nd and 5th) of animals the concentration of serum, urea, Creatinine, Uric acid, Total protein and Urine Urea, uric acid, creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating (group 3, 4 & 6, 7) with ethanol extract of *A.reticulata* showed significant decrease ($p<0.001$) in concentration of serum urea , Creatinine , Uric acid , Total protein and Urine Urea,

uric acid, creatinine compared to gentamicin treated groups (2nd and 5th).

Considerably decrease in activity of SOD and glutathione peroxidase in gentamicin treated animals (2nd and 5th) when compared to normal animals (group 1). Treating (group 3, 4 & 6, 7) with ethanol extract of *A. reticulata* significantly prevented decrease in the level of SOD, GPx activity compared to gentamicin treated rats (2nd and 5th). Nevertheless considerable increase in activity of lipid peroxidase in gentamicin treated animals (2nd and 5th). Treating (group 3, 4 & 6, 7) with ethanol extract of *A.reticulata* prevented increase in the level of lipid peroxidase. Thus strongly inhibit lipid peroxidation in isolated tissue via its antioxidant activity. Gentamicin treated animals (2nd and 5th) developed a significant damage observed as elevated serum levels of specific enzymes like SGPT, SGOT and when compared to normal control. Treating (group 3, 4 & 6, 7) with ethanol extract of *A. reticulata* showed good protection against gentamicin induced toxicity.

There is significant ($p<0.01$) decrease in the RBC and WBC in control groups in comparison to the normal control group. However in the extract treated groups there is significant increase in RBC and WBC ($p<0.01$ for ethanol extract).

In gentamicin treated group of animals weight of kidneys were considerable increased compared to normal animals (group1) and treating (group 3, 4 & 6, 7) with ethanol extract showed significant decrease ($p<0.001$) in kidney weight. There is significant ($p<0.01$) increase in the urine P^H and decrease in urine volume of the control groups in comparison to the normal control group. However in the extract treated groups there is significant reduction in the urine ph ($p<0.05$ for ethanol extract) and increase in urine volume and results were showed in table -2,3,4,5 and 6.

In Cisplatin Induced Model

In Cisplatin treated groups (2^{nd} and 5^{th}) of animals the concentration of serum urea, Creatinine, Uric acid, Total protein and Urine Urea, uric acid, creatinine were considerably increased than the normal animals (group 1) which indicates severe nephrotoxicity. Treating (group 3, 4 & 6, 7) with ethanol extract of *A.reticulata* showed significant decrease ($p<0.001$) in concentration of serum urea , Creatinine , Uric acid , Total protein and Urine Urea, uric acid ,creatinine compared to Cisplatin treated groups (2^{nd} and 5^{th}).

Considerably decrease in activity of SOD and glutathione peroxidase in cisplatin treated animals (2nd and 5th) when compared to normal animals (group 1). Treating (group 3, 4 & 6, 7) with ethanol extract of A. ret iculata significantly prevented decrease in the level of SOD, GPx activity compared to gentamicin treated rats (2nd and 5th). Nevertheless considerable increase in activity of lipid peroxidase in cisplatin treated animals (2nd and 5th). Treating (group 3, 4 & 6, 7) with ethanol extract of *A.reticulata* prevented increase in the level of lipid peroxidase. Thus strongly inhibit lipid peroxidation in isolated tissue via its antioxidant activity.

Cisplatin treated animals (2nd and 5th) developed a significant damage observed as elevated serum levels of specific enzymes like SGPT, SGOT and when compared to normal control. Treating (group 3, 4 & 6, 7) with ethanol extract of *A.reticulata* showed good protection against cisplatin induced toxicity.

There is significant ($p<0.01$) decrease in the RBC and WBC in control groups in comparison to the normal control group. However in the extract treated groups there is significant increase in RBC and WBC ($p<0.01$ for ethanol extract).

In Cisplatin treated group of animals weight of kidneys were considerably increased compared to normal animals (group1) and treating (group 3 , 4 & 6,7) with ethanol extract showed significant decrease ($p<0.001$) in kidney weight. There is significant ($p<0.01$) increase in the urine ph and decrease in urine volume of the control groups in comparison to the normal control group. However in the extract treated groups there is significant reduction in the urine ph ($p<0.05$ for ethanol extract) and increase in urine volume. There is significant ($p<0.01$) decrease in the body weights control groups in comparison to the normal control group. However in the extract treated groups there is significant increase in body weight ($p<0.01$ for ethanol extract) and results were showed in table -7,8,9,10 and 11.

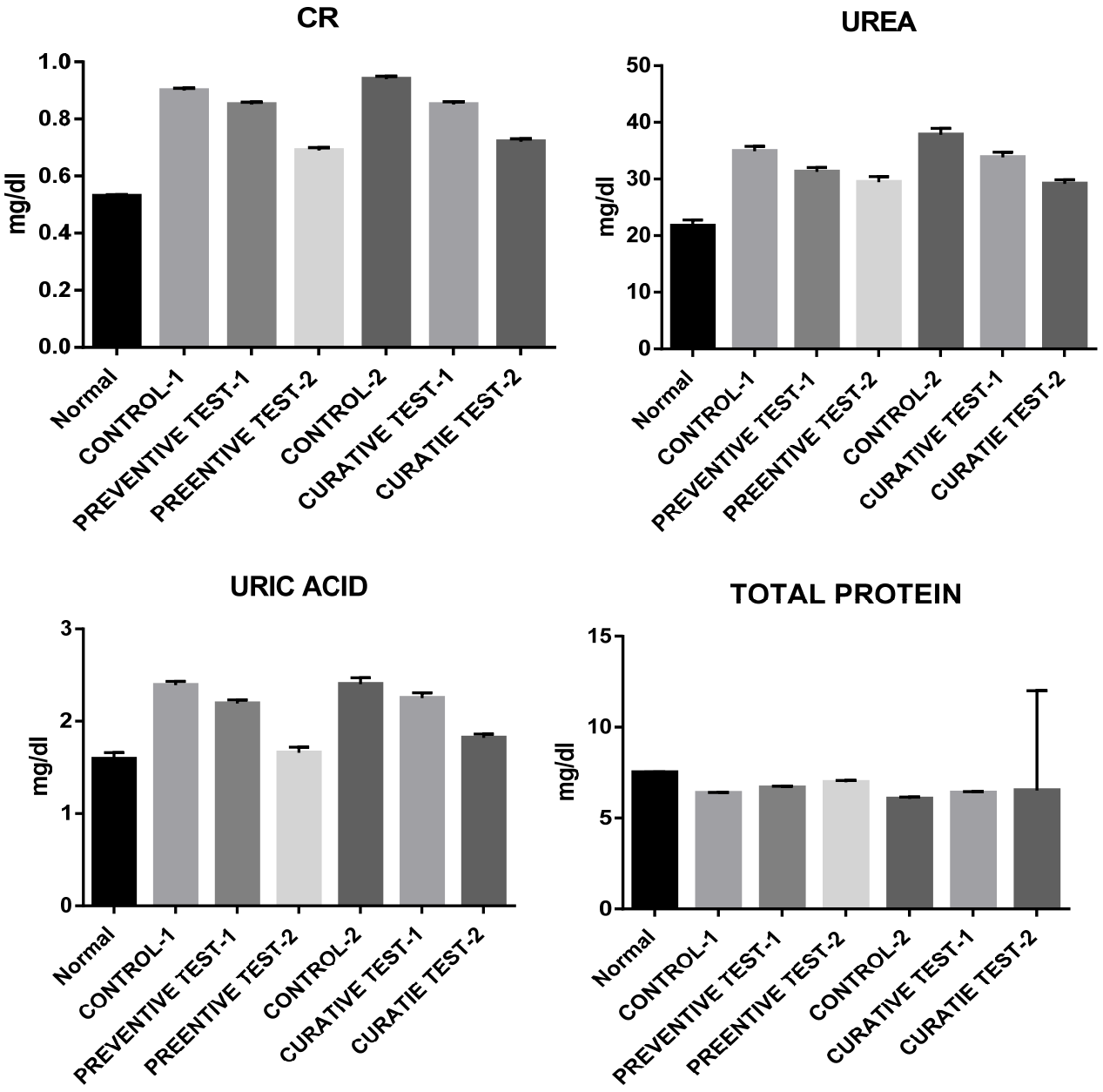
Table 1 Preliminary Phytochemical Analysis:

Phytoconstituents	Present or Absent
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Coumarins	+
Potein & amino acids	+
Saponins	+
Tannins	-
Phenolic compounds	+
Flavonoids	+
Alkaloids	+

+ indicates present and – indicates absence

Table 2: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* oral *on* serum creatinine, urea, uric acid and Total protein treated in rats for 24 days -Gentamicin Model

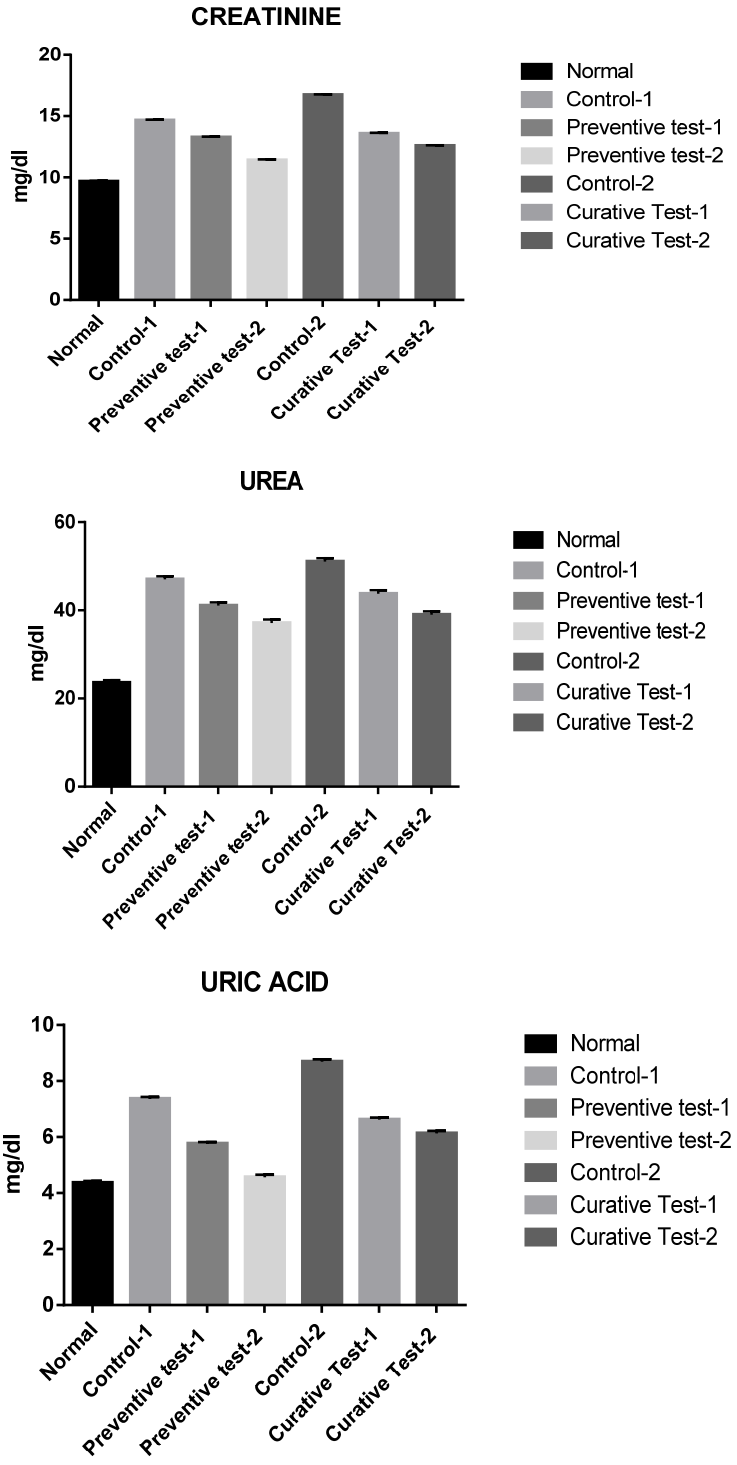
Group	Drug Treatment	Creatinine	Urea	Uric acid	Total protein
Normal		0.53±0.005	21.73±1.03	1.59±0.07	7.50±0.03
Control-1	40mg/kg	0.9±0.008***	34.98±0.87***	2.39±0.04***	6.37±0.03***
Preventive test-1	250mg/kg	0.85±0.009***	31.29±0.74***	2.19±0.04***	6.67±0.08***
Preventive test-2	500mg/kg	0.69±0.01***	29.45±0.97***	1.66±0.06	6.97±0.10**
Control-2	40mg/kg	0.945±0.01***	37.8±1.13***	2.40±0.07***	6.06±0.10***
Curative Test-1	250mg/kg	0.851±0.01***	33.8±0.91***	2.25±0.06***	6.38±0.07***
Curative Test-2	500mg/kg	0.725±0.01***	29.145±0.73***	1.82±0.04	6.52±5.49***



Graph 1, 2, 3& 4: Effect of extract on Serum Biochemical Parameters (Gentamicin model)

Table 3: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* oral *on* Urine creatinine, urea, and **uric acid treated** in **rats for** 24 **days -Gentamicin Model**

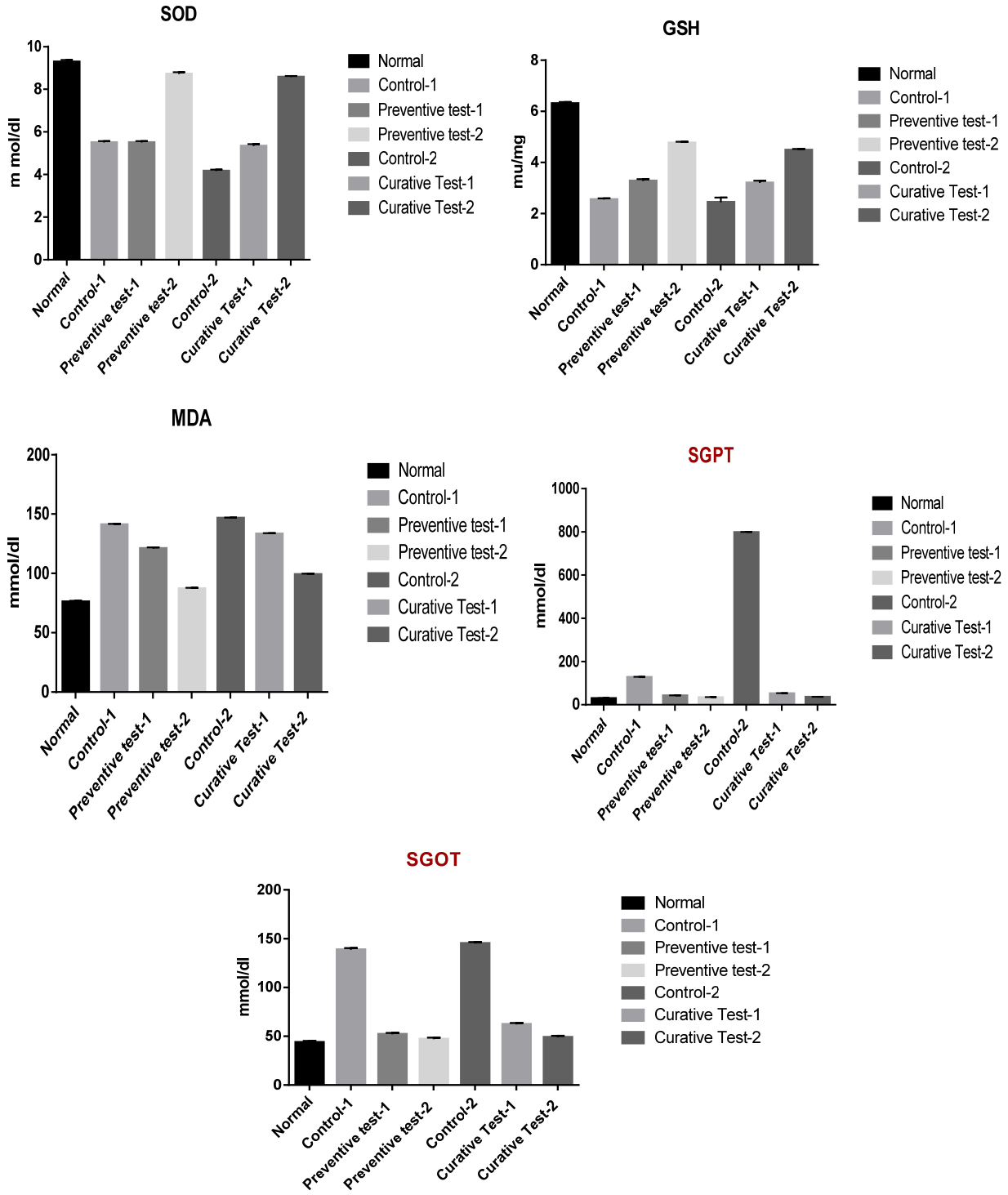
Group	Drug Treatment	Creatinine	Urea	Uric acid
Normal		9.66±0.06	23.59±0.58	4.36±0.07
Control-1	40mg/kg	14.65±0.06***	47.02±0.71***	7.36±0.07***
Preventive test-1	250mg/kg	13.25±0.08***	41.07±0.76***	5.76±0.06***
Preventive test-2	500mg/kg	11.40±0.08***	37.13±0.81***	4.56±0.09
Control-2	40mg/kg	16.72±0.06***	51.06±0.78**	8.69±0.08***
Curative Test-1	250mg/kg	13.56±0.08***	43.77±0.80***	6.62±0.08***
Curative Test-2	500mg/kg	12.57±0.06***	39.05±0.75***	6.13±0.09**



Graph 5, 6 & 7: Effect of extract on Urine Biochemical Parameters (Gentamicin model)

Table 4: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* orally on treated in rats for 24 days on antioxidant and Liver parameters Gentamicin Model

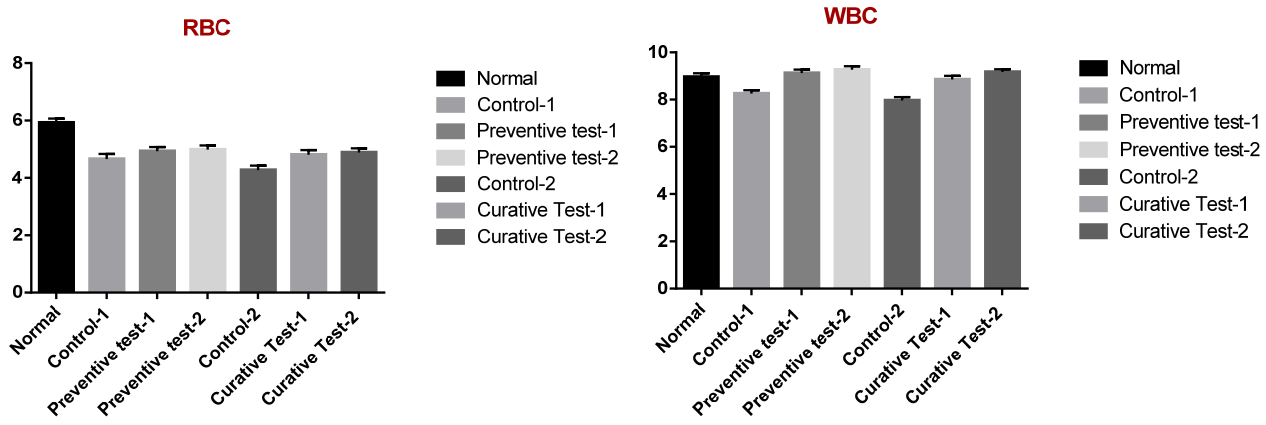
Groups	Treatment(mg/kg)	SOD	GSH	MDA	SGPT	SGOT
I.		9.29±0.09	6.30±0.06	76.05±0.8	29.9±1.34	43.63±1.62
I.	40	5.50±0.07***	2.55±0.05***	141.00±0.71***	127.8±1.42***	138.66±1.78***
I.	250	5.50±0.07***	3.28±0.07***	121.15±0.71***	42.3±1.41**	51.88±1.50**
/.	500	8.72±0.08**	4.76±0.05**	87.08±0.78**	33.85±1.72	46.98±1.56*
/.	40	4.16±0.07***	2.44±0.19***	146.54±0.62***	797.85±1.45***	145.15±1.48***
I.	250	5.34±0.09***	3.20±0.09***	133.17±0.81***	52.21±1.68**	62.01±1.51***
I.	500	8.58±0.05**	4.48±0.05***	99.04±0.76***	35.78±1.70	48.95±1.47**



Graph 8-12: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* orally on treated in rats for 24 days on antioxidant and Liver parameters -Gentamicin Model

Table 5: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulate* oral on RBC/WBC treated in rats for 24 days -Gentamicin Model

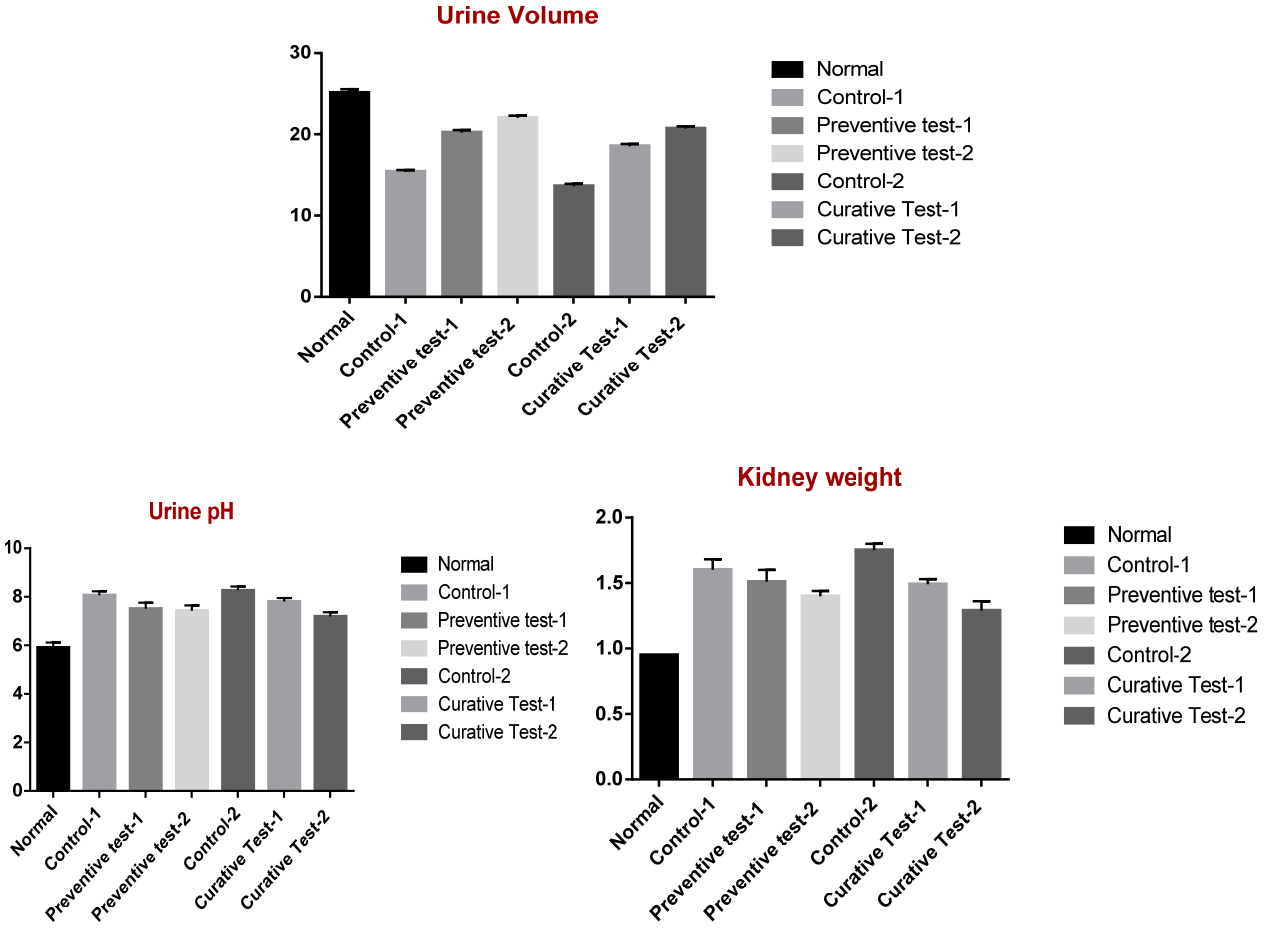
Group	Drug Treatment	WBC	RBC
Normal		8.96±0.15	5.92±0.15
Control-1	40mg/kg	8.25±0.15**	4.66±0.18**
Preventive test-1	250mg/kg	9.12±0.15	4.93±0.15
Preventive test-2	500mg/kg	9.26±0.15*	4.98±0.15*
Control-2	40mg/kg	7.96±0.14***	4.27±0.16***
Curative Test-1	250mg/kg	8.85±0.15	4.81±0.16
Curative Test-2	500mg/kg	9.17±0.12	4.88±0.15



Graph 13 & 14: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* oral *on* RBC&WBC

Table 6: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* orally on Body weight, Urine volume, Urine Ph and Kidney weight treated in rats for 24 days--Gentamicin Model

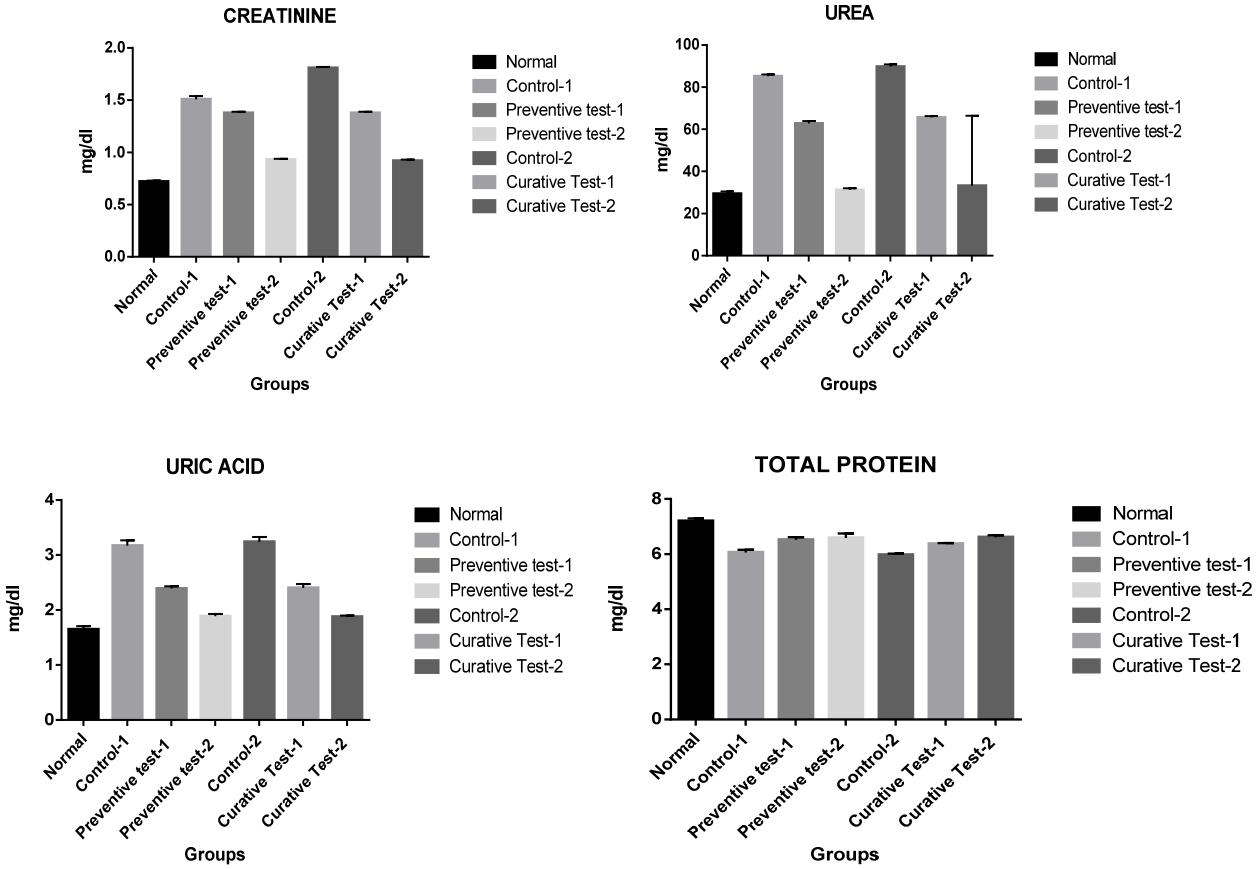
Group	Drug Treatment	Body weight	Urine volume	Urine pH	Kidney weight
Normal		186.66±0.88	25.1±0.45	5.9±0.22	0.95±0.01
Control-1	40mg/kg	150.66±1.23***	15.39±0.22*	8.06±0.17***	1.60±0.08**
Preventive test-1	250mg/kg	164.83±1.14***	20.2±0.34*	7.51±0.25*	1.51±0.09**
Preventive test-2	500mg/kg	172±0.96**	22.04±0.27	7.43±0.22**	1.40±0.04***
Control-2	40mg/kg	147.83±1.01***	13.61±0.30*	8.26±0.17***	1.75±0.05***
Curative Test-1	250mg/kg	162.5±0.76***	18.54±0.27*	7.8±0.15***	1.49±0.04***
Curative Test-2	500mg/kg	168.33±0.9***	20.72±0.26*	7.2±0.16***	1.29±0.07*



Graph 15, 16& 17: Effect of 40 mg/kg/day subcutaneous gentamicin and *A. reticulata* orally *on* Body weight, Urine volume, Urine Ph and Kidney weight treated in rats for 24 days-Gentamicin Model

Table 7: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* orally on serum creatinine, urea, uric acid and Total protein treated in rats for 16 days

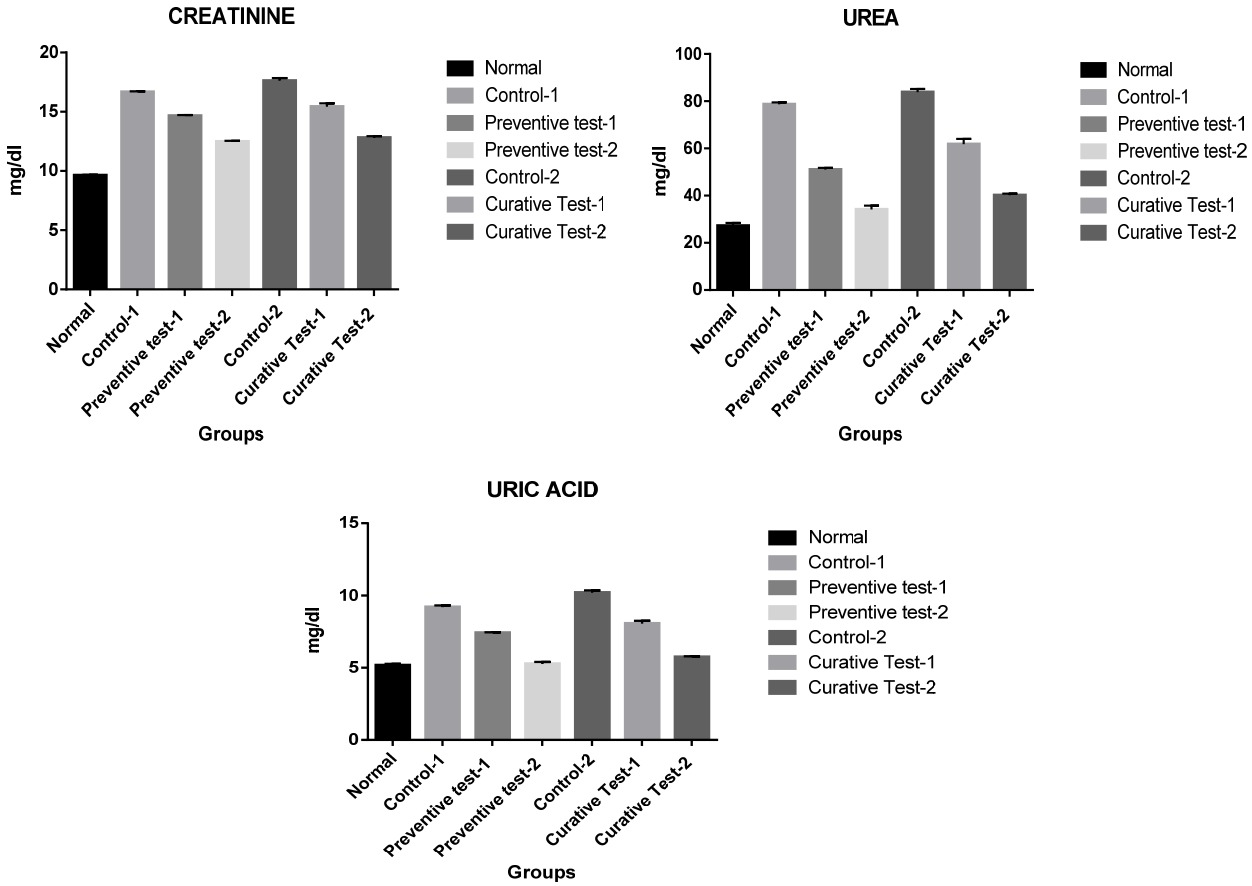
Group	Drug Treatment	Creatinine	Urea	Uric acid	Total protein
I.		0.72± 0.01	29.48± 0.96	1.65±0.06	7.2±0.1
I.	5 mg/kg	1.51± 0.03***	85.18± 0.90***	3.17±0.1***	6.06±0.1***
I.	250mg/kg	1.38± 0.01***	62.76±1.2***	2.39±0.04***	6.52±0.10***
/.	500mg/kg	0.93± 0.009***	31.26±0.75	1.89±0.04	6.59±0.16***
/.	5 mg/kg	1.81± 0.01***	89.79±1.10***	3.24±0.09***	5.97±0.05***
I.	250mg/kg	1.38± 0.009***	65.61±0.71***	2.40±0.07***	6.37±0.03***
I.	500mg/kg	0.92± 0.01***	33.21± 33.21*	1.88±0.02	6.61±0.07**



Graph 18, 19, 20& 21: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* orally on serum creatinine, urea, uric acid and Total protein treated in rats for 16 days

Table 8: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* orally on Urine creatinine, urea, and uric acid treated in rats for 16 days

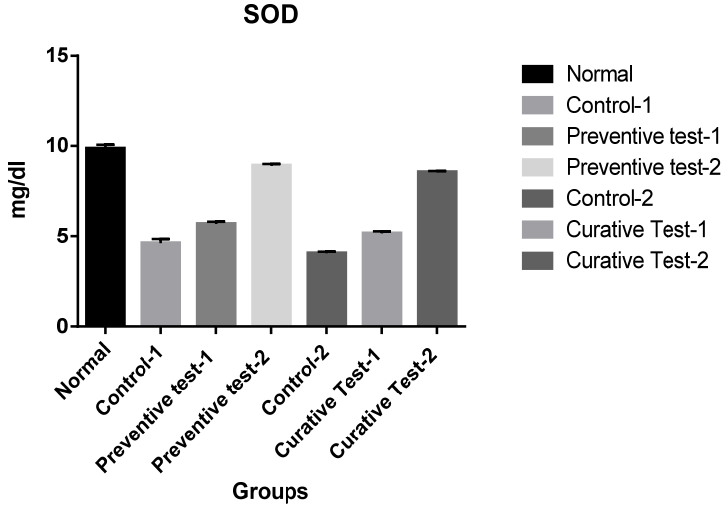
Group	Drug Treatment	Creatinine	Urea	Uric acid
I.		9.63±0.04	27.17±1.25	5.16±0.11
II.	5 mg/kg	16.68±0.04***	78.7±0.80***	9.2±0.12***
III.	250mg/kg	14.66±0.06***	51.0±0.75***	7.4±0.06***
IV.	500mg/kg	12.48±0.06***	34.13±1.67***	5.26±0.15
V.	5 mg/kg	17.61±0.23***	83.89±1.35***	10.2±0.16***
VI.	250mg/kg	15.43±0.27***	61.79±2.26***	8.05±0.21***
VII.	500mg/kg	12.8±0.14***	40.23±0.61***	5.73±0.06*

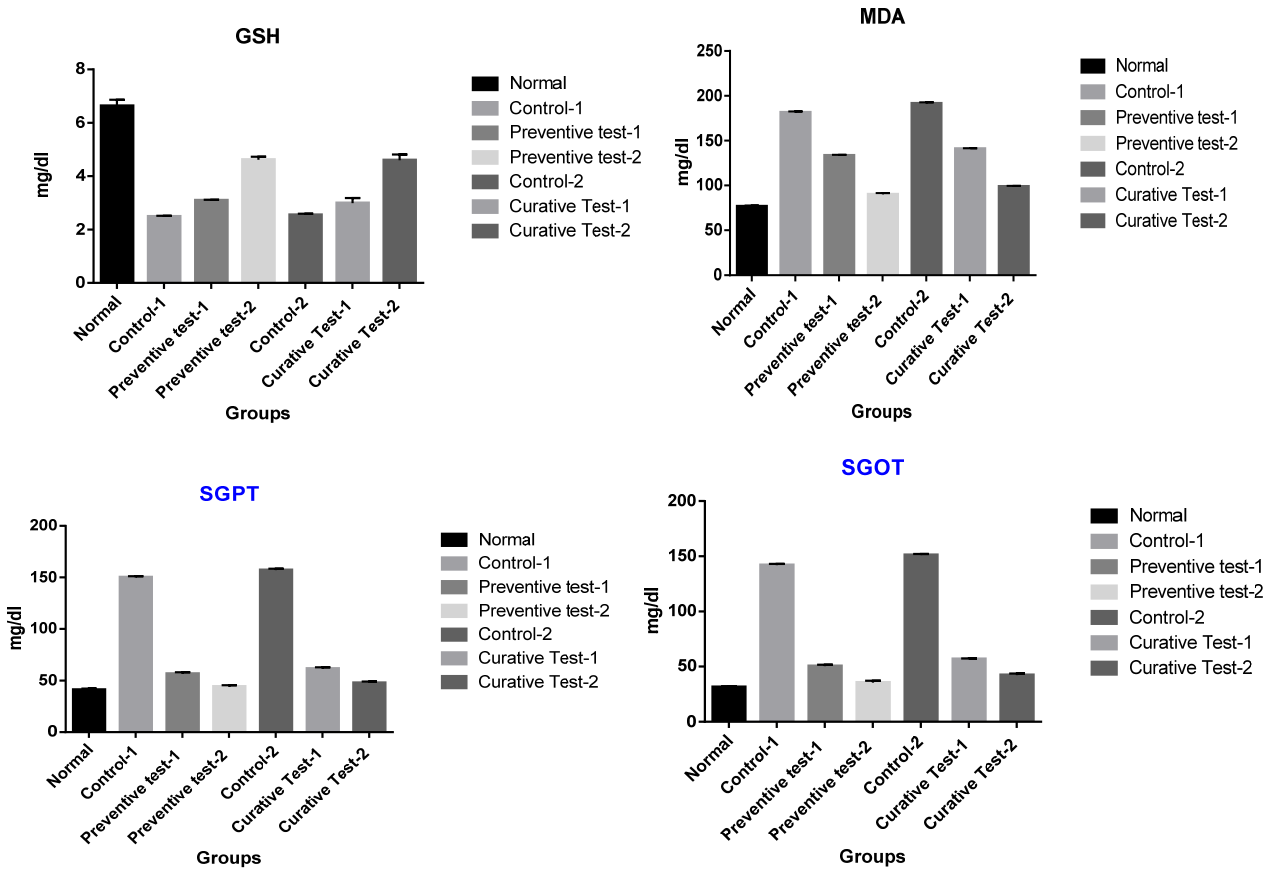


Graph 22, 23& 24: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* orally on Urine creatinine, urea, and **uric acid treated** in **rats for** 16 **days**

Table 9: Effect of 5 mg/kg/day subcutaneous cisplatin and *A. reticulata* orally on treated in **rats for** 16 **days**

Group	SOD	GSH	MDA	SGPT	SGOT
I.	9.85±0.22	6.63±0.23	76.84±0.68	41±1.32	31.41±0.76
I.	4.62±0.22***	2.49±0.03***	181.6±0.99***	150.1±1.1***	141.9±1.21***
I.	5.68±0.13***	3.09±0.03***	133.5±0.74***	56.6±1.54***	50.4±1.3***
/.	8.92±0.08***	4.62±0.11***	90.1±1.42***	44.1±1.5	35.4±1.9
/.	4.05±0.09***	2.55±0.05***	191.5±1.37***	157±1.57***	151±1.1***
I.	5.16±0.11***	2.99±0.19***	140.9±0.70***	61.5±1.4***	56.7±0.7***
I.	8.56±0.06***	4.60±0.22***	98.94±0.80***	47.8±1.3**	42.3±1.4***

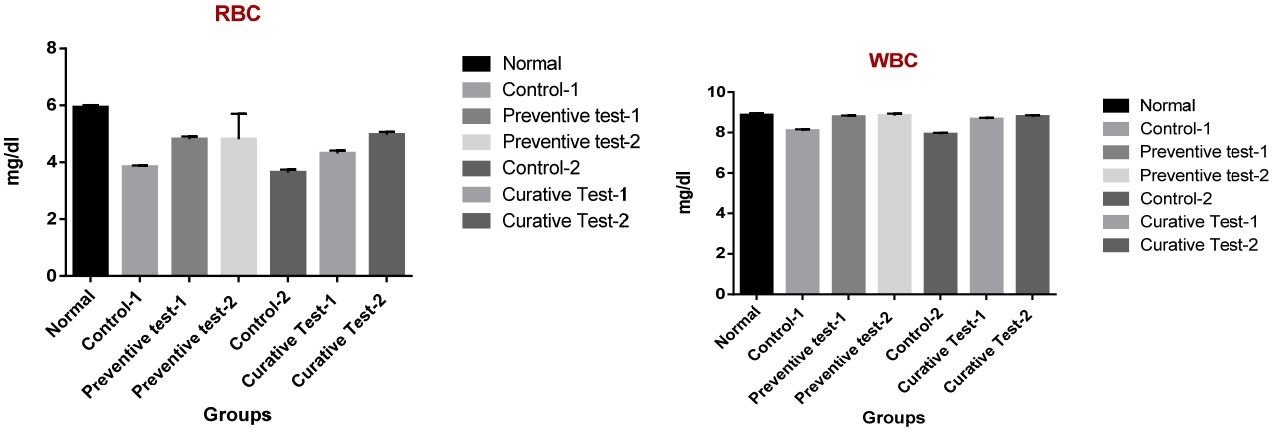




Graph 25, 26, 27, 28& 29: Effect of 5 mg/kg/day subcutaneous cisplatin and *A. reticulata* orally on treated in rats for 16 days

Table 10: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* oral on RBC/WBC treated in rats for 16 days

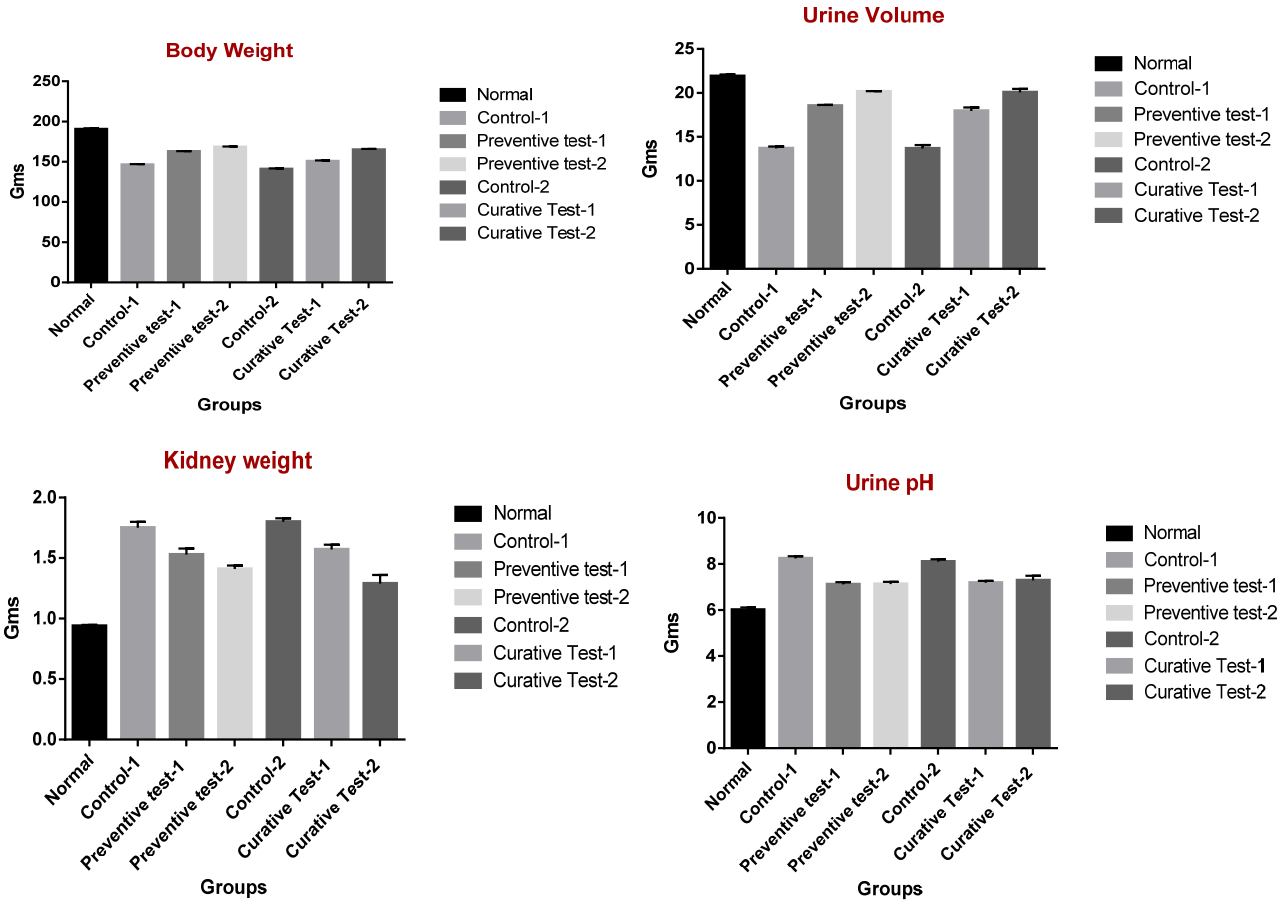
Group	RBC	WBC
I.	5.93±0.08	8.86±0.1
II.	3.84±0.05***	8.09±0.07***
III.	4.81±0.1***	8.78±0.07
IV.	4.81±0.9***	8.84±0.1
V.	3.64±0.1***	7.92±0.07***
VI.	4.31±0.1***	8.66±0.07
VII.	4.97±0.1***	8.79±0.07



Graph 30& 31: Effect of 5 mg/kg/day subcutaneous Cisplatin and *A. reticulata* oral on RBC& WBC treated in rats for 16 days

Table 11: Effect of 5 mg/kg/day subcutaneous CISPLATIN and Table :11 *A. reticulata* orally on Body weight, Urine volume, Urine Ph and Kidney weight treated in rats for 16 days

Group	Body weight	Urine volume	Urine pH	Kidney weight
I.	190.3±1.5	21.9±0.2	6.01±0.1	0.94±0.007
II.	146.1±1.0***	13.7±0.2***	8.24±0.1***	1.75±0.05***
III.	162.5±0.7***	18.54±0.1***	7.11±0.1***	1.53±0.05***
IV.	168.3±0.8***	20.16±0.03**	7.13±0.1***	1.41±0.03***
V.	140.8±1.0***	13.67±0.4***	8.1±0.1***	1.8±0.03***
VI.	150.6±1.2***	17.94±0.4***	7.19±0.08***	1.57±0.04***
VII.	164.8±1.2***	20.06±0.4**	7.29±0.2***	1.29±0.07***



Graph 32, 33, 34 & 35: Effect of 5 mg/kg/day subcutaneous cisplatin and *A. reticulata* orally on Body weight, Urine volume, Urine Ph and Kidney weight treated in rats for 16 days.

DISCUSSION

Gentamicin, aminoglycoside antibiotic with a wide spectrum of activities against Gram-positive and Gram-negative bacterial infections, with high preference for latter and cisplatin equally associate with nephrotoxicity as it is side effect[30-31].

Thus gentamicin and cisplatin induced nephrotoxicity is well established experimental model of drug induced renal injury [32-33]. Many animal experiments have demonstrated over the positive correlation between oxidative stress and nephrotoxicity[34].

Gentamicin and cisplatin induced nephrotoxicity by causing renal phospholipidosis through inhibition of lysosomal hydrolases such as sphingomyelinase and phospholipases in addition to causing oxidative stress.[35].

Drug induced nephrotoxicity are often associated with marked elevation in blood urea, serum creatinine and acute tubular necrosis[36]. So these biochemical parameters have been used to investigate drug induced nephrotoxicity in animals and man[37] in the present study drug induced nephrotoxicity were established by single daily intraperitoneal injection of the gentamicin, for 24 days and single administration of cisplatin for 16 days. This toxicity characterized by marked elevation in the circulating levels of blood urea, serum creatinine, uric acid, total protein and histological features of tubulonephritis in the model Control(group 2 and 5) rats when compared to untreated(group 1) rats. However these changes were attributed by pre-treatment with single daily graded doses of A.R extract for 24 days. Oral administration of plant extract significantly decreases the urea and creatinine, uric acid level in both treatment group compare to toxicant group in both models. Apart from the direct nephrotoxic effect of gentamicin and cisplatin in group 2 and 5 rats, the

acute elevation in the measured biochemical parameters could also be attributed to increased catabolic state of the rats due to the prolong anorexia associated with gentamicin and cisplatin nephrotoxicity.

In renal diseases, the serum urea accumulates because of the rate of serum urea production exceeds the rate of clearance[138]. Elevation of urea and creatinine levels in serum was taken as the index of nephrotoxicity[39-40]. Creatinine derives from endogenous sources by tissue creatinine breakdown114. Thus serum urea concentration is often considered a more reliable renal function prediction than serum creatinine. Anyhow the level of uric acid is non-significantly increased in the toxicant group when compared to control. Oral administration of plant extract significantly decreases the uric acid level in both treatment group compare to toxicant group.

It was established that gentamicin is actively transported into proximal tubules after glomerular filtration in a small proportion where it causes proximal tubular injury and abnormalities in renal circulation that leads to a reduction of GFR[41].

RBC, WBC count was decreased in control groups and in treatment groups there is increase in RBC and WBC compared to toxicant group

Physiological parameters body weight, urine volume was reduced in control groups and increased in treatment groups compared to control and vice versa with kidney weight, urine pH

In histopathological study of vehicle treated group showing some blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. Gentamicin treated group showing diffuse glomerular congestion, Tubular casts, Peritubular congestion, epithelial desquamation, Blood vessel congestion. While in treatment group (500 mg/kg, Group IV) shows focal glomerular congestion, Peritubular congestion, Focal hydrophic degeneration of tubular epithelial cells and treatment group (500 mg/kg, Group VII) shows only some of the blood vessels are dilated and congested within the interstitium. Also few scattered mononuclear inflammatory infiltration is seen within the interstitium. From histopathological results we can conclude that A.R extract at dose of 500 mg/kg (Preventive) have partial protective effect while A.R extract at dose of 500 mg/kg (curative) have protective effect on gentamicin induced nephrotoxicity.

The findings suggest that the potential use of ethanol extract of *Annona reticulata* therapeutically used as a nephroprotective agent. Therefore further studies to explain their mechanisms of action should be conducted to aid the discovery of new therapeutic agents for the treatment of renal diseases.

CONCLUSION

On evaluating biochemical parameters it was found that the ethanolic extract of aerial parts of *Annona reticulata* showed nephro-protective activity in both Gentamycin and cisplatin induced nephrotoxicity the models due to presence of therapeutic phyto constituents.

ACKNOWLEDGMENT:

The authors are thankful to the Principal, Chairman and management of Shri Vishnu College of Pharmacy,Vishnupur, Bhimavaram, for providing the facilities for conducting the stud

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