EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF 14- DEOXY-11,12 DIDEHYDRO ANDROGRAPHOLIDE IN DRUG INDUCED NEPHROTOXICITY

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Abstracts : Gentamycin are Aminoglycoside antibiotic have been used widely for the treatment of infections caused by gram-negative organisms. But eventually results in severe adverse effects namely nephrotoxicity or renal disorder through generation of reactive oxygen species (ROS).the existing drug can cure most of the disease.stil there is a never ending search for finding new drug in the hope that it would yield drug with lesser side effects and better therapeutic benefit than the existing drugs. this work was researched for evaluation of the Nephroproctive activity of 14- deoxy-11,12 Didehydro Andrographolide against gentamicin induced nephroprotective the 14- deoxy-11,12 Didehydro Andrographolide administered orally (10, 15,20mg/kg) for 8 day Gentamicin was administered at the dose of 7 mg/kg for 1st day intraperritoneally .Gentamicin treated group showed increased level of serum &Urine Creatinine uric acid urea , which were significantly retrived in group pretreated with 14- deoxy-11,12 Didehydro Andrographolide .in conculsion theHistopathological & Biochemical parameters Confirmed that the 14- deoxy-11,12 Didehydro Andrographolide protect against Gentamicin induced renal damage , probably through its antioxidant activity.

Key Words: 14-Deoxy -11,12 Didehydro Andrographolide, gentamicin, Nephrotoxicity.

Introduction : The kidney is an essential organ required by the body to perform several important functions including the maintenance of homeostasis, regulation of the extracellular environment, such as detoxification, and excretion of toxic metabolites and drugs[1,2] Therefore, the kidney can be considered as a major target organ for exogenous toxicants. Nephrotoxicity is a kidney-specific feature in which excretion does not go smoothly owing to toxic chemicals or drugs. Approximately 20% of nephrotoxocity is induced by drugs, but medication of the elderly increases the incidence of nephrotoxicity up to 66% as the average life span increases. Chemotherapy or anticancer medicine has been of limited use due to nephrotoxicity [3,4]. Nephrotoxicity can be diagnosed through a simple blood test. Evaluation of nephrotoxicity through blood tests includes the measurements of blood urea nitrogen (BUN), concentration of serum creatinine, glomerular filtration rate and creatinine clearance. However, these assessments of nephrotoxicity are only possible when a majority of kidney function is damaged. Therefore, discovery and development of biomarkers that can detect kidney dysfunction at the early stage are needed. In this research, we summarize the mechanisms of 14-Deoxy-11,12 Didehydro and rographolid as nephroprotective and highlight their constituent in protective action.

Material And Method :

Animal : The healthy Wistar albino rats of either sex weighing between 150-200 g were taken for the study they were housed under controlled conditions of temperature (23 plus minus 2 degree Celsius, humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water approved by the institutional Animal Ethical Committee as per the CPCSEA guidelines.

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Drug &Chemical : 14- Deoxy-11,12 Didehydro

Andrographolide Purchased from Natural Remedies Private Limited ,Gentamicin was purchased From Cipla And other reagents were Purchased and all chemicals used in these studies were of analytical grade.

Experiments design : Experimental were performed in accordance with the guidelines for the care and use of laboratory animals laid down by the committee for the purpose of control and supervision of experiments in animal (CPCSA) The rats were divided into six group of six each . Group 1 (control) received distilled water (1ml/kg po),for 8 day.group-2 (toxic)received Gentamicin (7mg/kg i.p) for 8 day. Group-3 received Gentamicin (7mg/kg i.p) and Cystone (std,75mg/kg p.o)(standard group) for 8 days .Group 4,5, and 6 received 10,15,20mg/kg 14- Deoxy-11,12 Didehydro Andrographolide (p.o) (Test-1, Test-2,and Test-3) for 8 day in addition to this,the animal in groups 4,5,and 6 were co administrated intraperitoneally once in 1st day with Gentamicin in a Dose of 7mg/kg[6]

Estimation of different parameters : At the end of the experiment on 8th day the animals were anaesthetized with anesthetic ether and sacrificed . the blood sample were collected from retro- orbital plexus and left at room temperature for 2h The blood sample were centrifuged for 10 minutes at 3000 rpm to separate the serum .the sera were estimated for concentration of serum Creatinine urea ,uric acid and urine for creatinine urea ,uric acid and also serum &urine sodium, potassium were estimated for Nephroprotective activity. Kidney were removed for histopathological examination.

Histopathtological studies of rat kidneys.

Kidneys of sacrified animals were identified and carefully desected out for histopathological study after rinsing in normal saline section. The tissue was fixed in 10% formal saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 m thick section stained with haematoxyllin-eosin and fition under a photomicroscope(magnification power-40x).

Statistical analysis : Arithmetic means of the values of readings were calculated for each experiment the results obtained were used for statistical analysis using INTA software. The data obtained from various models of nephrotoxicity in rats were subjected to analysis of variance(ANOVA)Followed by Dunnetts t-test using INTA software. Value of p less than5%

I.e. $P<0.05$ was considered statistically significant.

Result – body and kidney weight there is significant decrease in the body weight &significant increase in kidney weight of toxic groups observed when compared to normal group, where as the significant decrease in kidney weight showed in the standard,test 1,test 2 &test 3(table no.1)

Table -1 Effects on Body weight, kidney weight in normal, Gentamicin, and 14- Deoxy- 11,12- didehydro andrographolide treated rats

Group No.	Treatment	Body Weight (gm)	Kidney Weight (gm)
1	Vehicle	206.80±1.629	1.255±0.01618
2	Gentamicin	188.08±1.532##	1.502±0.01292##
3	Gentamicin + Cystone	204.60±2.305**	1.295±0.01347**
4	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide10mg/kg	190.53±1.428ns	1.481±0.01121ns
5	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide15mg/kg	197.45±2.255*	1.422±0.01134**
6	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide20mg/kg	205.46±1.546**	1.303±0.01542**

N=6, values are expressed as Mean± SEM, comparison were made as follows,# $p<0.05$,## $p<0.01$ when compared with normal control,* $p<0.05$,** $p<0.01$ when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.

Urine volume –there is significant decrease in urine volume of toxic group when compare to normal group where as insignificant increase the test 1 group & in standard, test 1 & test 2 there is significant in the urine volume of rats when compared to toxic group(table no.2)

Urine analysis:there is insignificant increase in urine urea,creatinine (table no.2)&uric acid (table no.3) in toxic group when compared to normal group,where as test 1 showed the insignificant decrease in urine urea creatinine (table no.2) &uric acid (table no.3)as compare to toxic group & significant increase in the standard, test 2 &test 3 groups were compared to toxic group. there is significant decrease & increase in urine sodium & potassium respectively in the toxic group when compare to normal group, & significant increase & decrease in urine sodium, potassium respectively in the test first group as compare to toxic group where as the significant increase in urine sodium & significant decrease urine potassium in the standard,test 1,& second group (table no.3)

Table- 2Effects on urine volume, urine urea, urine creatinine in normal, Gentamicin, and 14- Deoxy- 11,12-didehydro andrographolide treated rats.

Group No.	Treatment	Urine Volume	Urine urea	Urine creatinine (mg/dL)
1	Vehicle	2.938±0.1485	32.540±1.423	0.3593±0.01467
2	Gentamicin	1.592±0.1240##	55.713±1.202##	0.8903±0.0986##
3	Gentamicin + Cystone	2.571±0.1146**	34.492±1.436**	0.1373±0.0560**
4	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide10mg /kg	1.836±0.1392ns	52.920±1.377ns	0.3202±0.1307ns
5	Gentamicin + 14- Deoxy-11,12-didehydro andrographolide 15mg/kg	2.154±0.0768*	48.013±0.875**	0.0988±0.0403**
6	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide 20mg/kg	2.349±0.1146**	35.977±1.074**	0.1718±0.0701**

N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.

Table-3 Effects on,urine uric acid, Na+ and K+ in normal, Gentamicin, and 14- Deoxy -11,12-didehydro andrographolidetreated rats.

Group No.	Treatment	Urine Uric acid (mg/dL)	Na+ (meq/L)	K+ (meq/L)
1	Vehicle	5.440±0.1588	145.74±1.315	5.360±0.108
2	Gentamicin	12.427±0.1503##	133.45±1.387##	9.978±0.090##
3	Gentamicin + Cystone	6.052±0.1267**	143.78±1.463**	5.782±0.081**
4	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide10mg /kg	10.763±0.0779**	134.44±1.398ns	9.218±0.194**
5	Gentamicin + 14- Deoxy-11,12-didehydro andrographolide 15mg/kg	9.754±0.1140**	139.29±0.849**	9.035±0.120**
6	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide 20mg/kg	6.146±0.1452**	142.76±1.346**	5.946±0.235**

N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.

There is insignificant increase in serum urea, creatinine & uric acid in toxic group when compare to normal group, where as test 1 showed the insignificant decrease in serum urea, creatinine &significant increase in uric acid as compare to toxic group & significant increase in the standard, test 2& test 3 group when compare to toxic group (table no.4)

There is significant decrease & increase in serum sodium & potassium respectively in the toxic group when compare to normal group, & insignificant increase & decrease in serum sodium, potassium respectively in the test 1 group as compare to toxic group where as the significant increase in serum sodium & significant decrease serum potassium in the standard, test 1, test 2 group (table no.5)

Table-4 Effects on serum urea, serum creatinine, serum uric acid, Na+ and K+ in normal, Gentamcin, and 14- Deoxy-11,12-didehydro andrographolidetreated rats.

Group No.	Treatment	Serum Urea (mg/dL)	Serum Creatinine (mg/dL)	Serumuric acid (mg/dL)
1	Vehicle	34.906±1.693	0.945±0.1112	2.819±0.1418
2	Gentamicin	55.714±1.732##	1.969±0.1191##	6.593±0.1431##
3	Gentamicin + Cystone	38.627±1.673**	1.056±0.1112**	3.220±0.1158**
4	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide10mg /kg	51.911±1.411ns	1.865±0.1159ns	6.153±0.1354ns
5	Gentamicin + 14- Deoxy-11,12-didehydro andrographolide 15mg/kg	47.082±1.891**	1.365±0.1277**	5.737±0.1147**
6	Gentamicin + 14- Deoxy- 11,12-didehydro andrographolide 20mg/kg	37.555±1.148**	1.157±0.1131**	3.025±0.1752**

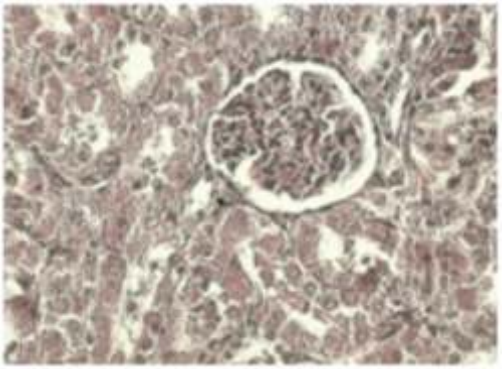
N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.

Table-5 Effects on Na+ and K+ in normal, Gentamicin, and 14- Deoxy 11,12-didehydro andrographolidereated rats.

Group No.	Treatment	Body Weight (gm)	Kidney Weight (gm)
1	Vehicle	150.42±1.273	5.145±0.1097
2	Gentamicin	134.68±1.332##	6.270±0.1285##
3	Gentamicin + Cystone	147.64±1.135**	5.354±0.1433**
4	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide10mg/kg	137.48±1.185ns	6.001±0.2287ns
5	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide15mg/kg	140.46±1.509*	5.582±0.1143*
6	Gentamicin + 14- Deoxy - 11,12-didehydro andrographolide20mg/kg	147.61±1.234**	5.417±0.1309**

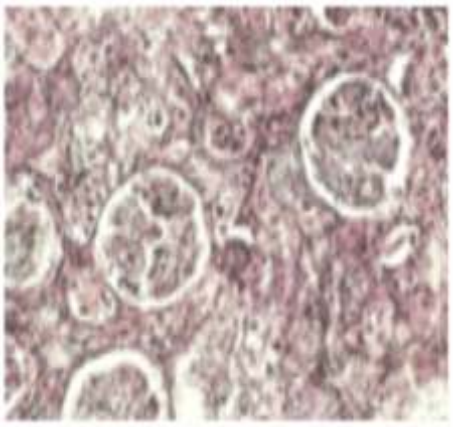
N=6,values are expressed as Mean± SEM, comparison were made as follows,# p<0.05,## p<0.01 when compared with normal control,* p<0.05,** p<0.01 when compared with negative control(values are compared on 15th day by one way ANOVA Dunnett t test) N.S.= not significant.

Group-I : Male albino rats with intake of normal distilled water showed normal architecture of renal glomeruli with intact bowmans capsule. Brush bordered cuboidal epithelium lining the proximal convoluted tubules Simple cuboidal epithelium lining the distal convoluted tubules. macula densa is very prominent (fig.a).



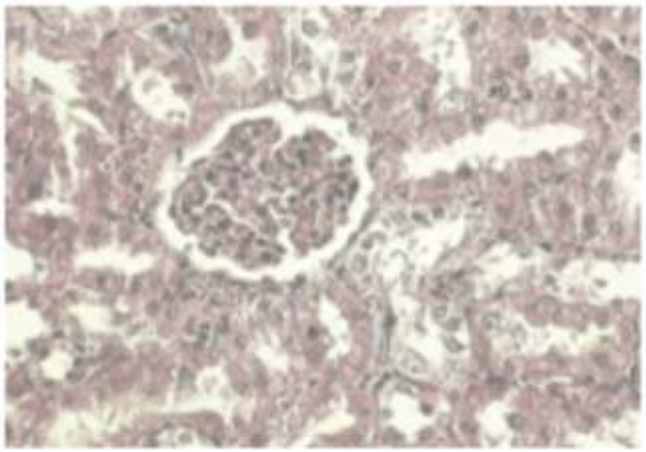
Group No.1 (Vehicle Conrtol)

Group-II : In the negative control groupII, histopathological findings showed kidney structure distorted by severe necrosis of tubules. The stroma was edematous. The tissue was infiltrated by numerous chronic inflammatory cells. Engorged blood vessels and areas of hemorrhage were seen. Features suggested severe tubular necrosis. Renal histology in the cisplatin treated group showing severe tubular necrosis(fig.b).



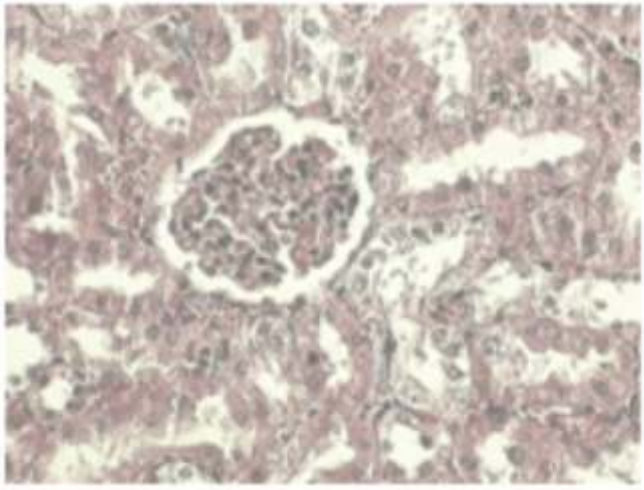
Group-2 (Gentamicin)

Group-III : In the group III, histopathological findings showed the stroma with a mild degree of edema. moderate degree of congestion was also seen in the glomeruli. Numerous engorged blood vessels were seen. Mild tubular changes were noted. The tissue was free from inflammatory cells Renal histology in the A groupIII showing moderate tubular necrosis with significant reversal of inflammatory changes.(fig.c).



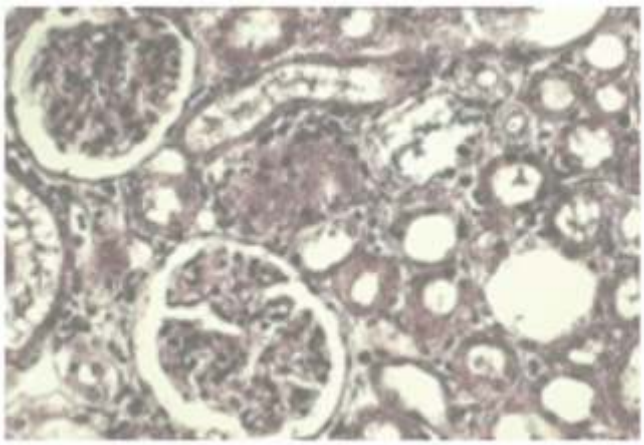
Group-3 (Gentamicin+Cystone)

Group-IV : In the group IV, histopatological examination showed that there was mild interstitial edema. moderate degree of congestion was also seen in the glomeruli. Numerous engorged blood vessels were seen. Mild tubular changes were noted. The tissue was free from inflammatory cells Renal histology in the A groupIV showing moderate tubular necrosis with significant reversal of inflammatory changes (fig.d).



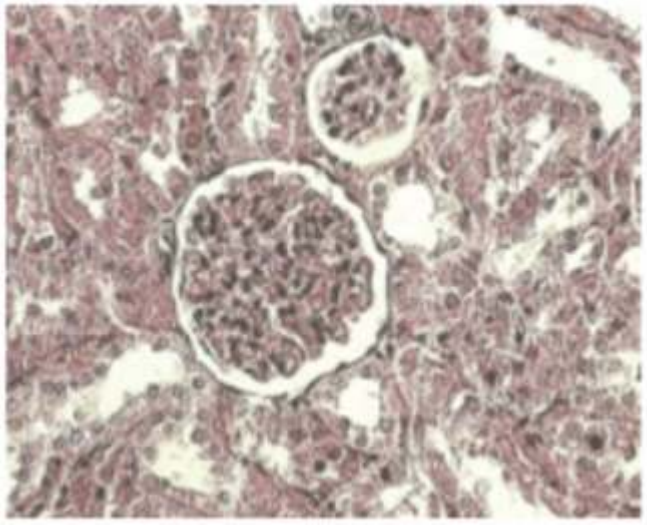
Group-4 (Gentamicin+14- Deoxy-11,12 Didehydro Andrographolide10mg/kg)

Group-V : In group V, histopathological findings showed mild interstitial edema, mild degree of peritubular and glomerular congestion and numerous engorged blood vessels. The tissue was free from inflammatory cells. Features suggested mild tubular changes (fig.e).



Group-5 (Gentamicin+14- Deoxy-11,12 Didehydro Andrographolide15mg/kg)

Group-VI : In group VI histopathology showed mild interstitial edema, mild degree of glomerular congestion and few congested blood vessels. Mild tubular damage was observed. The tissue was sparsely infiltrated by chronic inflammatory cells (fig.f).



Group-6 (Gentamicin+14- Deoxy-11,12 Didehydro Andrographolide20mg/kg)

Discussion : Gentamicin is a known nephrotoxic agent reported to induce a significant degree of nephrotoxicity at different dose levels. It's nephrotoxic potential was established at adose level of 80mg/kg in albino rats[5].

There was no change in the normal behavioral pattern of animals and no signs and symptoms of toxicity were observed and no mortality was observed till 24h.14- deoxy-11,12 Didehydro Andrographolide were safe up to a maximum dose of 20mg/kg body weight .the biological evaluation was carried out at doses of 10,15 and 20mg/kgb.w by oral route .urine urea, creatinine ,uric acid ,potassium ,and serum creatinine ,urea,uric acid ,potassium was found to be significant ($P<0.001$) increased in rats treated with only gentamicin ,whereas treatment with the 14- Deoxy-11,12 Didehydro Andrographolide reversed the effect of gentamicin indicating Nephroprotective activity (table No 1,2,3,4,5). The impairment in kidney functions is accompanied by an increase in urine &serum creatinine urea and uric acid level[6,7]. It is

one of the essential compounds for maintaining cell integrity participation in the cell metabolism .the significant and progressive weight loss in gentamicin the 14- Deoxy-11,12 Didehydro Andrographolide showed dose depended protective effect. 14- Deoxy-11,12 Didehydro Andrographolide might have exhibited nephroprotective activity by the virtue of its antioxidant activity.[8]

Conclusion : Taking into consideration the results obtained in the present investigation , it can be concluded that 14- Deoxy-11,12 Didehydro Andrographolide has a definite Nephroprotective activity :hence it could be use in the treatment of kidney disorder like kidney dysfunction.

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