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AN EVALUATION OF TREATMENT MODALITY OF MANDALIDAMSA WITH AJITAGADA IN EXPERIMENTAL LEVEL

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Abstract

It is proposed to study the Renal-protective effect of *Ajitagada yoga* in Spague-dawley rats. All the animals were sacrified and kidney was used for the histopathological studies. Serum creatinine level is used as an index of nephrotoxicity. The present study was undertaken to assess the nephroprotective effect of Ajitagada against gentamicin induced nephrotoxicity in Sprague-dawley rats. Eight adult female Sprague-dawley rats weighing 160-300g, the renal protective effect of Ajitagada' was conducted at Veterinary college & sciences, Mannuthi, Thrissur.

INTRODUCTION:

The fatal cases of poisoning are poisonous snakebites. In India it is believed that snakes bite about 2 million people annually, of which 15,000-30,000 cases prove fatal. There are around 3000 species of snakes in the world of which 200 are found in India. Colubridae and Viperidae families of venomous snakes are of medical importance in India and in Kerala. Numbers of incidence of bites are more from Viper and Pit viper bites are not rare. No antivenom is being made against Indian Pit vipers. Systemic involvement has been hardly reported and symptomatic treatment is the existing mode in Modern system of medicine.Research is a scientific study to establish and analyse facts to contribute to the present knowledge.

MATERIALS AND METHODS:

Experimental animals: The study was conducted in 40 adult female Sprague Dawley rats weighing 200 – 300 g.The rats were purchased from Small animal breeding station, Mannuthy. The animals were housed in appropriate cages in a well ventilate room with a 12-h light; 12-h dark cycle. They were maintained under identical feeding and management practices in the laboratory. An acclimatization period of four days was allowed before the commencement of the experiment. The experiment was conducted for a period of 30 days.

Plant material/Drug: The whole plant of Boerhavia diffusa was collected from the campus of college of Veterinary and animal sciences, Mannuthy and identified.

Preparation of aqueous extract of Boerhavia diffusa: The whole plant of Boerhavia diffusa was air dried at room temperature and coarsely powdered using an electrical pulverizer. 100g of the powder was mixed with 1 liter of distilled water and kept undisturbed for 24 hours. Then they were subjected to boiling for 30 minute with constant stirring. The extracts were filtered through a muslin cloth and then kept in boiling water bath for the complete evaporation of water.

Gentamicin sulphate: Gentamicin sulphate (procured from TTK pharma limited, Raja Annamalaipuram, Chennai, India) was administered at a dose rate of 80mg/kg intra peritoneal for eight days to induce Nephrotoxicity.

Experimental design: The animals were randomly divided into 5 groups comprising eight animals each. The experiment was conducted for a period of 30 days (Actually 15 days are correct time for this study but I extended upto 30 days for observations).Eight rats were retained as healthy control. Rests of the rats were treated with Gentamicin sulphate at a dose rate of 80 mg/kg intra peritoneal for 8 days.

Group 1 – Healthy control

Group 2 – Gentamicin sulphate was given at a dose rate of 80mg/kg intra peritoneal for eight consecutive days and administered with vehicle

Group 3 - Aqueous extract of Ajitagada was administered at a dose rate of 200 mg/kg p.o. from 9^{th} day to day 30.

Group 4 - Aqueous extract of Ajitagada was administered at a dose rate of 400 mg/kg p.o. from 9^{th} day to day 30.

Group 5 - Aqueous extract of Boerhavia Diffusa was administered at a dose rate of 400 mg/kg p.o. from 9^{th} day to day 30.

The blood was collected from all the animals on 0^{th}, 9^{th}, 15^{th}, and 30^{th} Day and serum was separated and used for the estimation of creatinine, urea, albumin and total protein.

On 30^{th} day, the rats were sacrified and both the kidneys were located and dissected out. The kidneys were

immediately weighed and used for conducting histopathological studies.

Collection of biological samples- The blood was collected from all the animals on 0^{th}, 9^{th}, 15^{th} and 30^{th} day and serum was used for the estimation of creatinine, urea, albumin and total protein.

Collection of blood and separation of serum

Blood was collected from the retro-orbital plexus under mild Diethyl ether anaesthesia with heparinized capillary tubes, into sterile centrifuge tubes without adding any anticoagulant. It was kept at refrigeration temperature for half an hour, taken out and kept at room temperature for another half an hour. It was then centrifuged at 3200 rpm for 10 minutes and the clear serum obtained was pipette out.

Kidney: The animals were euthanized and dissected upon and the kidney was collected. It was washed in running tap water to remove the blood clots and kept in chilled 0.9 percent sodium chloride.

OBSERVATIONS

Estimation of Serum parameters

Creatinine: Creatinine in serum was determined based on jaffe kinetic method without deprotienisation in 'Blood-analyzer' using Creatinine test kit from Agappe diagnostics limited, india.

Clinical Significance: It is formed in muscles from phosphor creatinine. It is important form of energy being a stor of high energy phosphate. Creatinine determinations have one advantage over urea determination that it is not affected by a high protein diet. Serum creatinine is more specific and sensitive indicator of renal function simultaneous estimation of serum Urea and Creatinine provides better information. Serum urea nitrogen, creatinine ratio is > 15 in pre renal failure and < 10 in renal failure.

Principle: Creatinine reacts with picric acid to produce a coloured compound, Creatinine alkaline picrate. The change in absorbance is proportional to the Creatinine concentration.

Reagents:

Reagents 1: Creatinine dye reagent
Picric acid - 8.73 mmol/L Surfactant
Reagent 2: Creatinine base reagent
Sodium hydroxide- 300 mmol/L
Sodium phosphate- 25 mmol/L
Reagent 3: Creatinine standard
Creatinine standard concentration 2 mg/dL

Procedure

	Standard	Sample
Working Reagent	1000µL	**1000 µL**
Standard	100 µL	-
Sample	-	**100 µL**
Mix and read the value of sample through the Blood-analyzer		

Normal value of Serum Creatinine in Sprague-dawley rat –0.2 – 0.8 mg/dl

UREA

Clinical significance: Proteins cannot be stored in human body, so excess should be broken down. Amino acids which form the components of proteins break down to give ammonia. This is toxic and so through a series of chemical reactions (urea cycle) non toxic urea is produced which is released into the blood excreted in the urine.

Elevated levels are seen during increased protein breakdown, dehydration, vomiting,

and diarrhoea. Also seen in any kind of renal disorder like Glomerular nephritis, chronic nephritis and nephritic syndrome.

Principle

Enzymatic determination of urea according to the following reaction.

Urea + H2O urease= 2NH3 + CO2

NH3 + Salicylate = nitroprusside hypo chloride 2.2 – Dicarboxy Indophenols

Reagents:

Reagents 1: Urea – B Colour reagent R1

Sodium Salicylate 80 nmol/L

Sodium nitroprusside 4 mmol/L

Sodium hypochloride 45 MG/Dl

Reagent 2: Urea – B R2

Phosphate buffer, (Ph 6.9) 60 mmol/L

Urease- 25 KU/L

Reagent 3 : Urea – B standard

Urea – B standard concentration 40 mg/dL

Procedure:

	Blank	Standard	Sample
Working Reagent	1000µL	1000µL	**1000µL**
Standard	-	10 µL	-
Sample	-	-	**10 µL**
Mix and incubate 5 min. at 37C then add			
Colour reagent	1000µL	1000µL	**1000µL**
Mix and incubate 5 min. at 37C then add			
DI Water	1000µL	1000µL	**1000µL**
Mix well and measure the absorbance of sample and the standard against the reagent blank			

After that with the help of this formula, we can convert the Urea value into BUN value. So I got BUN value for observation.

BUN = UREA VALUE x 0.46

Normal value of Serum BUN in Sprague-dawley rat –15 – 21 mg/dl

TOTAL PROTIEN

Clinical significance: Protien forms the major portion of dissolved substancesm,in the plasma. They form the basic structural components of the body. They constitutes the enzymes present in our body and also act as source of energy. The other functions include distribution of water, buffering, transport of various components, defence and coagulation of blood in our body. Increased levels are found in dehydration and myeloma. Decreased levels are found in liver disorders, Nephrotic syndrome, malnutrion and protein losses due to haemorrhage.

Principle: Colorimetric determination of total protein based on the principle of the Biuret reaction (copper salt in alkaline medium). Protien in plasma or serum sample forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

REAGENT COMPOSITION

Total protein reagent

Potassium iodide 6 mmol/L

Potassium sodium tartarate 21 mmol/L

Copper sulphate 6 mmol/L

Sodium hydroxide -58 mmol/L

Total protein standard

Standard concentration 6 gm/dl

Procedure

	Blank	Standard	Sample

Reagent	1000µL	1000µL	**1000µL**
Standard	-	20 µL	-
Sample	-	-	**20 µL**
Mix and incubate for 10 min. at 37C. Measure the absorbance of sample and the standard against the reagent blank			

Normal value of Serum Total protien in Sprague-dawley rat –5.6 – 7.6 mg/dl

ALBUMIN

Clinical significance: Albumin which is synthesized in the liver constitutes a Major part of the total proteins in the body, the other part being globulin; they form the major portion of the dissolved substances in the plasma. Functions of Albumin includes distribution of extracellular fluid, regulation of osmotic pressure, acts as a transport agent for a wide variety of substances such as hormones lipids, vitamins etc. Increased levels are seen in dehydration. Decreased levels are seen in liver diseases (Hepatitis, Cirrhosis), malnutrition, kidney disorders, and increased fluid loss during extensive burn and malabsorption.

Principle: The reaction between albumin from serum or plasma and the dye bromocresol –green produces a change in colour that is proportional to the albumin concentration.

REAGENT COMPOSITION

Albumin reagent

Succinate buffer, (Ph 4.20) 75 mmol/L
Bromocresol green 0.14 g/L
Copper sulphate 6 mmol/L
Sodium hydroxide 58 mmol/L

Albumin standard

Standard concentration 3 gm/dl

Procedure

	Blank	**Standard**	**Sample**
Reagent	1000µL	1000µL	**1000µL**
Standard	-	10 µL	-
Sample	-	-	**10 µL**
Mix and incubate for 1 min. at 37C. Measure the absorbance of sample and the standard against the reagent blank			

Normal value of Serum Albumin in Sprague-dawley rat –3.8 – 4.8 mg/dl

Histopathological examination of kidney

Representative samples of kidney obtained from the dissected animals were fixed in 10% formalin. They were then processed and paraffin embedded as described by Sheehan and Hrapchak, 1980. The sections were stained with haematoxylin and eosin as per the technique followed by Bancroft and cook, 1984. The sections were examined in detail under light microscope.

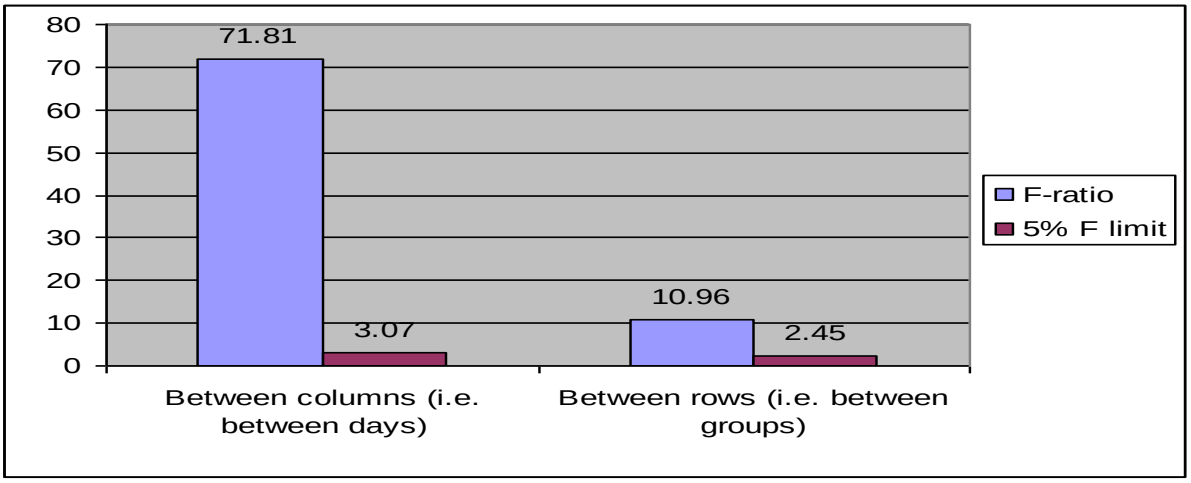
Statistical analysis of data: The results obtained were analysed using Analysis of covariance followed by Duncan's multiple range test for comparison between the groups as described by Snedecor and Cochran (1985). The cytoprotective enzymes were analysed using Analysis of variance (ANOVA). The best treatment from each group was selected and they were tested for significance using Student's t test.

Results

Creatinine;

Table No.1 the Anova table for the serum Creatinine values

Source of variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean square (MS)	F-ratio	5% F limit
Between columns (i.e. between days)	41.5	2	20.75	71.81	**3.07 (2,105)**
Between rows (i.e. between groups)	12.67	4	3.1675	10.96	**2.45 (4,105)**
Interaction	11.92	8	1.49	5.15	**2.02 (8,105)**
Within samples (Error)	30.34	105	0.288952		
Total	**96.43**	**119**			



The above table shows that all the three F-rations are significant of 5% level. This analysis does not support the null hypothesis which means there is too much difference in Creatinine values in different days (0^{th}, 9th & 15^{th}). Also different groups have too much difference.

Table No. 2 Comparison on Serum Creatinine values of 15^{th} day in between two group II & III.(Unpaired t - test)

Group	***Mean***	***SD***	**t - values**	***P values***
Group II	**0.75**	**0.3**	**0.22**	**P>0.05**
Group III	**0.71**	**.39**		

On comparing the values of the serum Creatinine on 15^{th} day, in between two groups, it was found that the mean score was 0.75 in Group II with SD + 0.3, and 0.71 with SD of + 0.39 in Group III. t values was found 0.22 which was statistically insignificant, P>0.05.

Table No. 3 Comparison on Serum Creatinine values of 15^{th} day in between two group II & IV. (Unpaired t - test)

Group	***Mean***	***SD***	**t - values**	***P values***
Group II	**0.75**	**0.3**	**1.36**	**P>0.05**

Group IV	**0.6**	**0.13**		

On comparing the values of the serum Creatinine on 15th day, in between two groups, it was found that the mean score was 0.75 in Group II with SD + 0.3, and 0.6 with SD of + 0.13 in Group IV. t values was found 1.36 which was statistically insignificant, P>0.05.

Table No.4 Comparison on Serum Creatinine values of 15th day in between two groups III & IV. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group III	**0.71**	**.39**	**0.77**	**P>0.05**
Group IV	**0.6**	**.13**		

On comparing the values of the serum Creatinine on 15th day, in between two groups, it was found that the mean score was 0.71 in Group III with SD + 0.39, and 0.6 with SD of + 0.13 in Group IV. t values was found 0.77 which was statistically insignificant, P>0.05.

Table No. 5 Comparison on Serum Creatinine values of 15th day in between two groups III & V. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group III	**0.71**	**.39**	**0.93**	**P>0.05**
Group V	**0.56**	**.25**		

On comparing the values of the serum Creatinine on 15th day, in between two groups, it was found that the mean score was 0.71 in Group III with SD + 0.39, and 0.56 with SD of + 0.25 in Group V. t values was found 0.93 which was statistically insignificant, P>0.05.

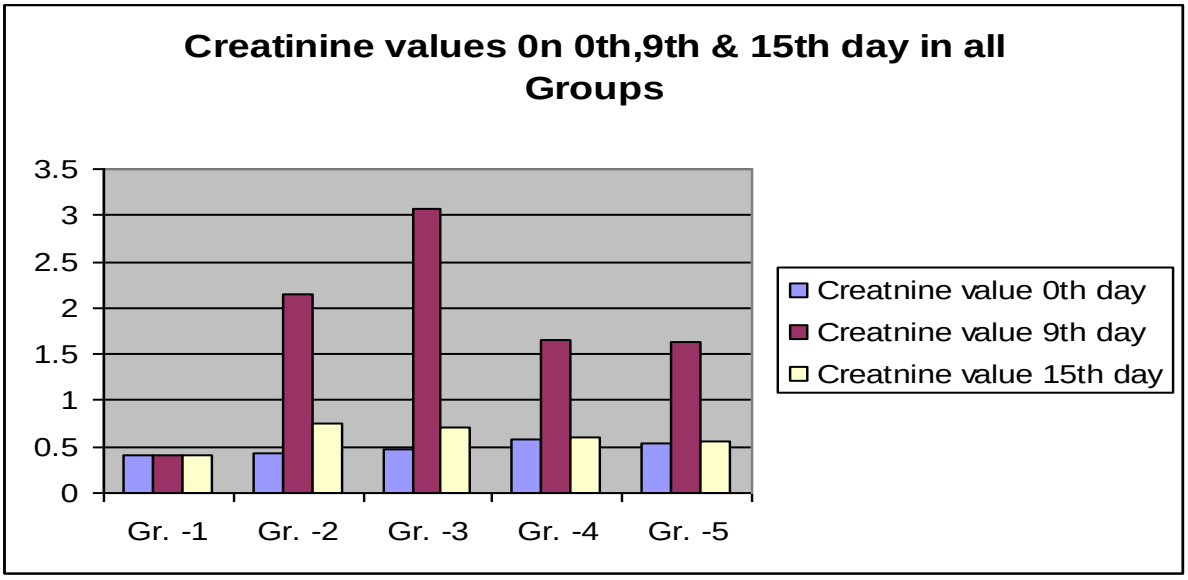
Table No. 6 Comparison on Serum Creatinine values of 15th day in between two group IV & V. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group IV	**0.6**	**.13**	**0.4**	**P>0.05**
Group V	**0.56**	**.25**		

On comparing the values of the serum Creatinine on 15th day, in between two groups, it was found that the mean score was 0.6 in Group IV with SD + 0.13, and 0.56 with SD of + 0.25 in Group V. t values was found 0.4 which was statistically insignificant, P>0.05.

Table No. 7 Comparison on Serum Creatinine values of all days in between all groups.

GROUP	0TH day (mean+SE)	9th day (mean+SE)	15th day (mean+SE)
I	0.41+0.06	0.41+0.04	**0.41+0.04**
II	0.42+0.05	2.14+0.37	**0.75+0.10**
III	0.47+0.05	3.06+0.51	**0.71+0.14**
IV	0.58+0.09	1.66+0.21	**0.6+0.05**
V	**0.54+0.08**	**1.64+0.13**	**0.56+0.09**



The results of the effect of Ajitagada on serum creatinine levels were presented in table. On Oth day, the mean serum creatinine values of groups I to V were 0.41+0.06, 0.42+0.05, 0.47+0.05, 0.58+0.09 and 0.54+0.08 mg/dl respectively.

On 9th day, all the groups except the normal group showed an increase in serum creatine values with mean values of 0.41+0.04, 2.14+0.37, 3.06+0.51, 1.66+0.21 and 1.64+0.13 mg/dl respectively for groups I, II, III, IV and V.

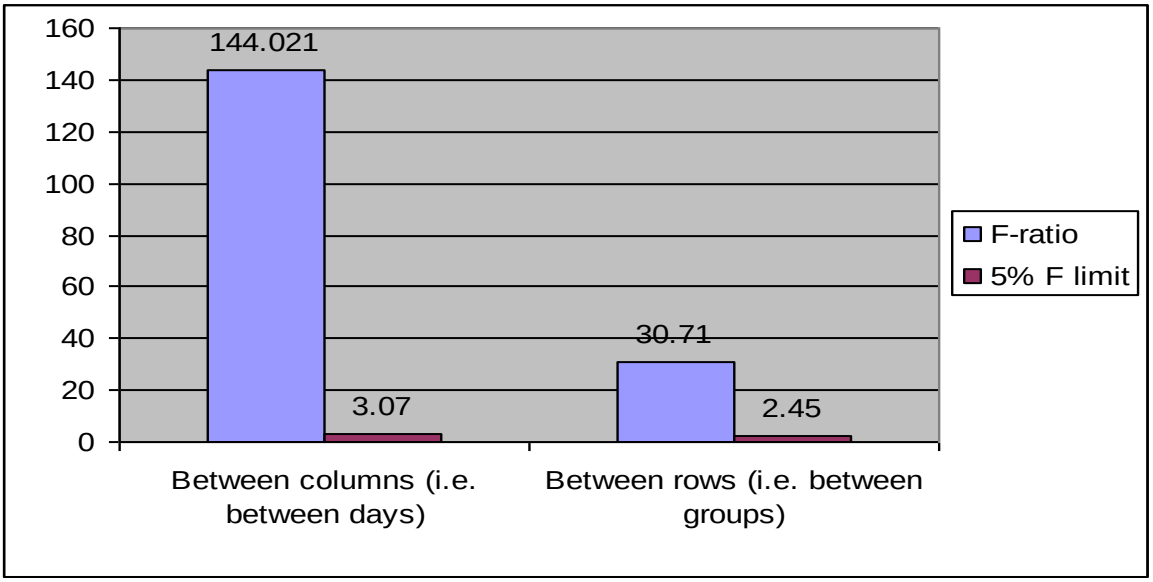
On 15th day, there was a significant reduction in serum creatinine values in groups III to V when compared with group II. The serum creatine values with mean values of 0.41+0.04, 0.75+0.10, 0.71+0.14, 0.6+0.05 and 0.56+0.09 mg/dl respectively for groups I, II, III, IV and V.

The result indicated that Ajitagada at a dose rate of 200 mg/kg showed considerable reduction in serum creatinine value than the other treatment groups.

BUN

Table No.8 the Anova test table for the serum BUN values

Source of variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean square (MS)	F-ratio	5% F limit
Between columns (i.e. between days)	232599.2	2	116299.6	144.021	**3.07 (2,105)**
Between rows (i.e. between groups)	99220.51	4	24805.13	30.71	**2.45 (4,105)**
Interaction	142834.2	8	17854.28	22.11	**2.02 (8,105)**
Within samples (Error)	84789.19	105	807.5161		
Total	**559443.2**	**119**			



The above table shows that all the three F-rations are significant of 5% level. This analysis does not support the null hypothesis which means there is too much difference in BUN values in different days (0^{th}, 9th & 15^{th}). Also different groups have too much difference.

Table No. 9 Comparison on Serum BUN values of 15^{th} day in between two groups II & III. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group II	**115.73**	**30.78**	**8.56**	**P>0.05**
Group III	**21..48**	**4.84**		

On comparing the values of the serum BUN on 15^{th} day, in between two groups, it was found that the mean score was 115.73 in Group II with SD + 30.78, and 21.48 with SD of + 4.84 in Group III. t values was found 8.56 which was statistically significant, P>0.05.

Table No. 10 Comparison on Serum BUN values of 15^{th} day in between two groups II & IV. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group II	**115.73**	**30.78**	**6.9**	**P>0.05**
Group IV	**32.54**	**14.55**		

On comparing the values of the serum BUN on 15^{th} day, in between two groups, it was found that the mean score was 115.73 in Group II with SD + 30.78, and 32.54 with SD of + 14.55 in Group IV. t values was found 6.9 which was statistically significant, P>0.05.

Table No. 11 Comparison on Serum BUN values of 15^{th} day in between two groups III & IV. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group III	**21..48**	**4.84**	**2.04**	**P>0.05**
Group IV	**32.54**	**14.55**		

On comparing the values of the serum BUN on 15^{th} day, in between two groups, it was found that the mean score was 21.48 in Group III with SD + 4.84, and 32.54

with SD of + 14.55 in Group IV. t values was found 2.04 which was statistically significant, P>0.05.

Table No. 12 Comparison on Serum BUN values of 15th day in between two groups III & V. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group III	**21..48**	**4.84**	**2.57**	**P>0.05**
Group V	**52.89**	**14.89**		

On comparing the values of the serum BUN on 15th day, in between two groups, it was found that the mean score was 21.48 in Group III with SD + 4.84, and 52.89 with SD of + 14.89 in Group V. t values was found 2.57 which was statistically significant, P>0.05.

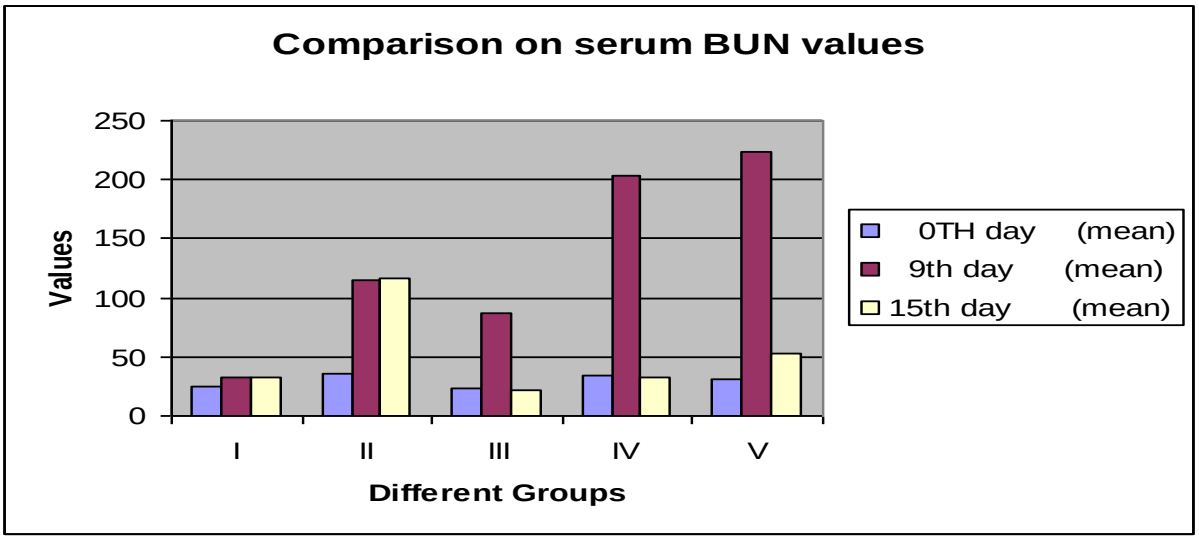
Table No. 13 Comparison on Serum BUN values of 15th day in between two groups IV & V. (Unpaired t - test)

Group	*Mean*	*SD*	t - values	*P values*
Group IV	**32.54**	**14.55**	**2.76**	**P>0.05**
Group V	**52.89**	**14.89**		

On comparing the values of the serum BUN on 15th day, in between two groups, it was found that the mean score was 32.54 in Group IV with SD + 14.55, and 52.89 with SD of + 14.89 in Group V. t values was found 0.22 which was statistically significant, P>0.05.

Table No. 14 Comparison on Serum BUN values of all days in between all groups.

GROUP	0TH day (mean+SE)	9th day (mean+SE)	15th day (mean+SE)
I	24.67+1.42	31.87+2.19	**32.02+1.96**
II	36.09+2.49	114.99+11.69	**115.73+10.77**
III	23.11+2.37	87.33+11.44	**21.47+1.69**
IV	34.36+3.802	203.11+27.59	**32.54+5.09**
V	**31.83+3.104**	**223.17+7.12**	**52.89+5.21**



The results of the effect of Ajitagada on serum BUN levels were presented in table.

On Oth day, the mean serum BUN values of groups I to V were 24.67+1.42, 36.09+2.49, 23.11+2.37, 34.36+3.802 and 31.83+3.104 mg/dl respectively.

On 9th day, all the groups except the normal group showed an increase in serum BUN values with mean values of 31.87+2.19, 114.99+11.69, 87.33+11.44, 203.11+27.59 and 223.17+7.12 mg/dl respectively for groups I, II, III, IV and V.

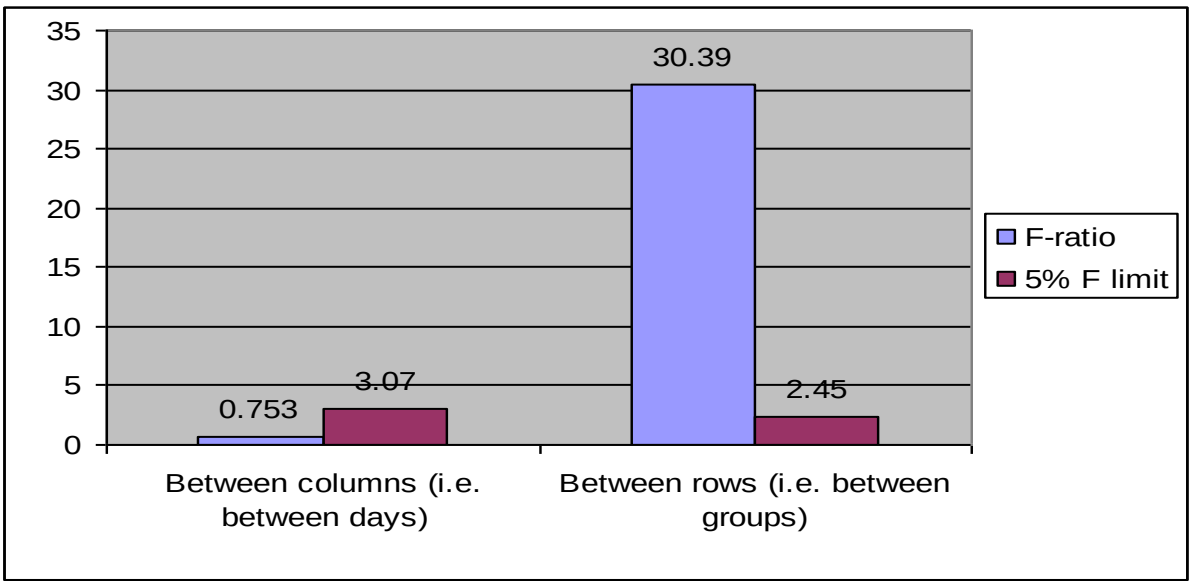
On 15th day, there was a significant reduction in serum BUN values in groups III to V when compared with group II. The serum BUN values with mean values of 32.02+1.96, 115.73+10.77, 21.47+1.69, 32.54+5.09 and 52.89+5.21mg/dl respectively for groups I, II, III, IV and V.

The result indicated that Ajitagada at a dose rate of 400 mg/kg showed considerable reduction in serum BUN value than the other treatment groups.

ALBUMIN

Table No.15 the Anova table for the serum Albumin values

Source of variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean square (MS)	F-ratio	5% F limit
Between columns (i.e. between days)	0.247	2	0.123	0.753	**3.07 (2,105)**
Between rows (i.e. between groups)	19.87	4	4.96	30.39	**2.45 (4,105)**
Interaction	.38	8	0.0475	.2289	**2.02 (8,105)**
Within samples (Error)	17.2	105	0.163		
Total	**37.69**	**119**			

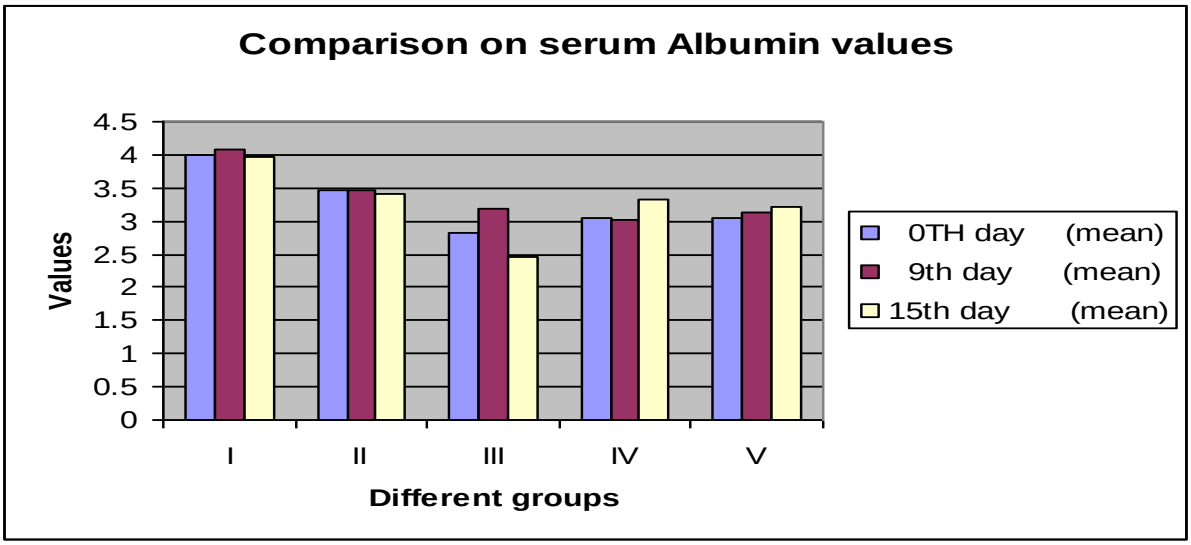


The above table shows that two F-rations are insignificant & F-rations are significant on between rows of 5% level. This analysis supports the null hypothesis in two places & does a not support in one place which means there is no difference in Abumin values in different days (0^{th}, 9th & 15^{th}) but different groups have differences due to chance.

Table No. 16 Comparison on Serum Albumin values of all days in between all groups.

GROUP	0^{TH} day (mean+SE)	9^{th} day (mean+SE)	15^{th} day (mean+SE)
I	4.01+0.14	4.08+0.13	3.96+0.17
II	3.46+0.13	3.46+0.08	3.42+0.09
III	2.81+0.18	3.18+0.09	2.46+0.12
IV	3.06+0.08	3.01+0.10	3.32+0.14
V	3.05+0.10	3.12+0.10	3.21+0.19

Means bearing the same superscript do not differ significantly at P<0.05



The results of the effect of Ajitagada on serum Albumin levels were presented in table.

On Oth day, the mean serum Albumin values of groups I to V Were 4.01+0.14, 3.46+0.13, 2.81+0.18, 3.06+0.08 and 3.05+0.10 mg/dl respectively.

On 9^{th} day, all the groups except the normal group showed an Increase in serum Albumin values with mean values of 4.08+0.13, 3.46+0.08, 3.18+0.09, 3.01+0.10 and 3.12+0.10 mg/dl respectively for groups I, II, III, IV and V.

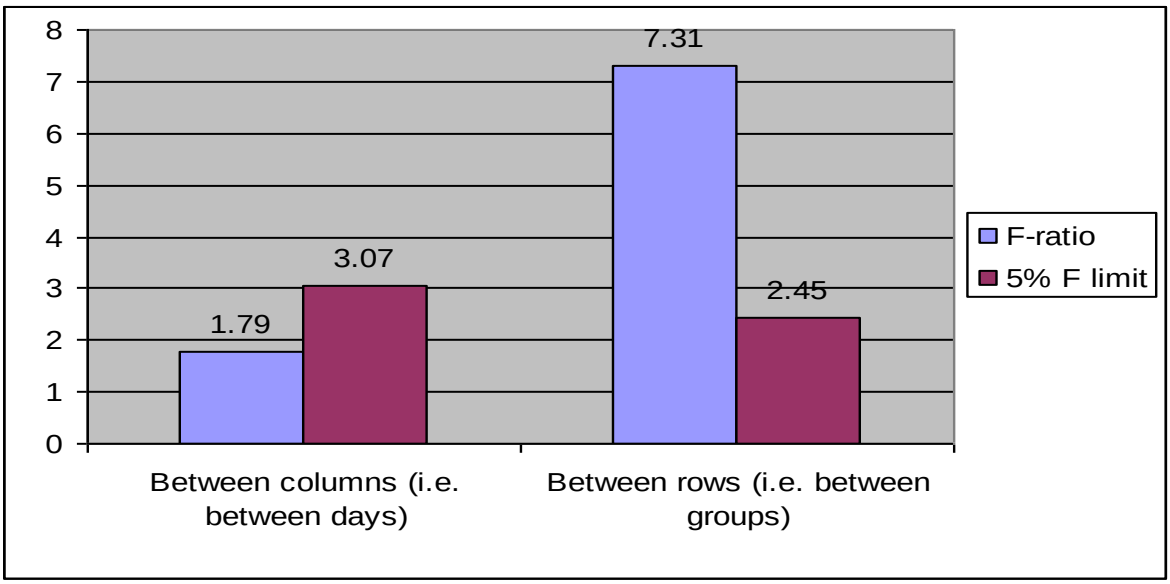
On 15^{th} day, there was a significant reduction in serum Albumin values in groups III to V when compared with group II. The serum Albumin values with mean values of 3.96+0.17, 3.42+0.09, 2.46+0.12, 3.32+0.14 and 3.21+0.19 mg/dl respectively for groups I, II, III, IV and V.

The result indicated that Ajitagada at a dose rate of 200 & 400mg/kg showed no considerable reduction in serum Albumin value than the other groups.

TOTAL PROTIEN

Table No.17 the Anova test table for the serum Total protien values

Source of variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean square (MS)	F-ratio	5% F limit
Between columns (i.e. between days)	0.729	2	0.365	1.79	**3.07 (2,105)**
Between rows (i.e. between groups)	5.95	4	1.49	7.31	**2.45 (4,105)**
Interaction	4025	8	503.13	2474.38	**2.02 (8,105)**
Within samples (Error)	21.35	105	0.203		
Total	**29.66**	**119**			

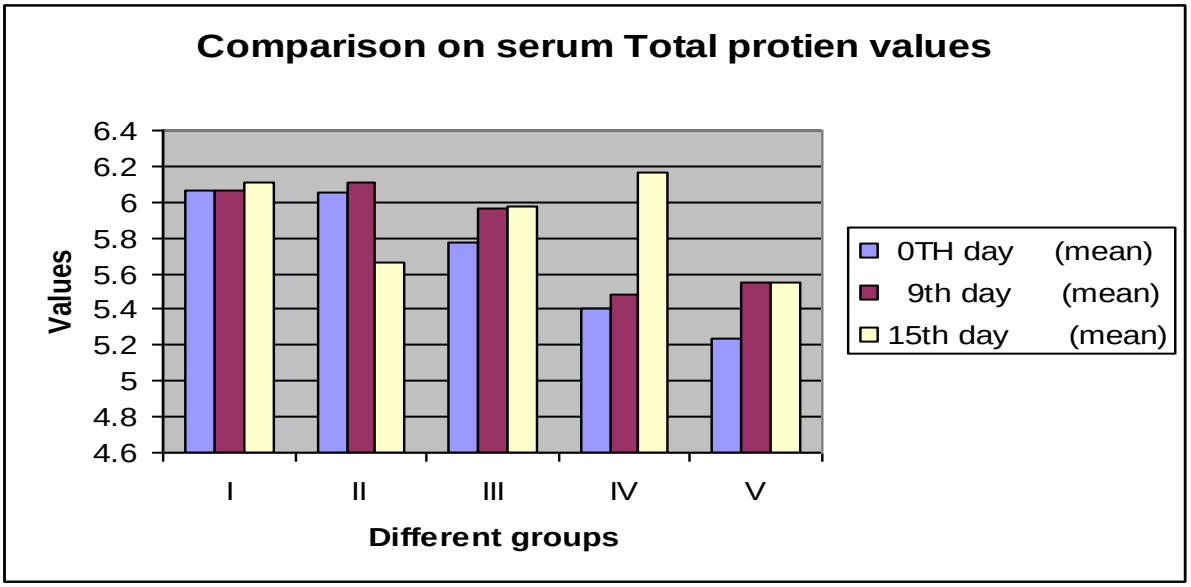


The above table shows that two F-rations are significant & F-rations are insignificant on between columns of 5% level. This analysis does not support the null hypothesis in two places & supports in one place which means there is no difference in Total protien values in different days (0^{th}, 9th & 15^{th}) but different groups have differences due to chance.

Table No. 18 Comparison on Serum Total protein values of all days in between all groups.

GROUP	0^{TH} day (mean+SE)	9^{th} day (mean+SE)	15^{th} day (mean+SE)
I	6.06+0.16	6.06+0.15	**6.11+0.17**
II	6.05+0.05	6.11+0.09	**5.66+0.07**
III	5.77+0.11	5.96+0.06	**5.98+0.23**
IV	5.41+0.09	5.48+0.08	**6.16+0.32**
V	**5.24+0.06**	**5.55+0.14**	**5.55+0.12**

Means bearing the same superscript do not differ significantly at P<0.05



The results of the effect of Ajitagada on serum Total protein Levels were presented in table.On Oth day, the mean serum Total protien values of groups I toV were 6.06+0.16, 6.05+0.05, 5.77+0.11, 5.41+0.09 and 5.24+0.06 mg/dl respectively.

On 9th day, all the groups except the normal group showed an increase in serum Total protien values with mean values of 6.06+0.15, 6.11+0.09, 5.96+0.06, 5.48+0.08 and 5.55+0.14 mg/dl respectively for groups I, II, III, IV and V.

On 15th day, there was a significant reduction in serum Total protien values in groups III to V when compared with group II. The serum Total protien values with mean values of 6.11+0.17, 5.66+0.07, 5.98+0.23, 6.16+0.32 and 5.55+0.12 mg/dl respectively for groups I, II, III, IV and V. The result indicated that Ajitagada at a dose rate of 200 & 400mg/kg showed no considerable reduction in serum Total protien value than the other groups. In other blood parameters like RBC, WBC, Neutrophil, Lymphocyte, Monocyte, Basophil, Eosinophil, there is no significant change in different days in all groups.

Histopathological examination of kidney

In normal group (group 1), the microscopic examination of the kidney revealed the usual histological parameters. The tubular structures were largely intact without the presence of any mononuclear infiltrates in the interstitial and congestion in the vessels.

In Gentamicin group (group 2), there were extensive proximal tubular necrosis and loss of the lining epithelium and regeneration features were predominantly sub capsular. Besides, there were interstitial oedema, perivascular oedema and multiple focal collections of mononuclear cells in the interstitial. The glomerular changes were quite marked (deffuse degenerative tubules).

In ajitagada 200mg/kg treated group (group 3), the Proximal tubular epithelial cells showed varying degrees of regeneration but slight degenerative changes. Besides this, there were scattered small foci of mononuclear cell infiltration confined to sub scapular area. The epithelial cells of the proximal convoluted

tubules were more or less intact. However, a few cells showed mild degenerative changes characterized by vacuolar cytoplasm. So kidney looks like healthy.

In Ajitagada 400mg/kg treated group (group 4), there were areas of tubular degeneration and necrosis along with areas of perivascular oedema and tubulo-interstitial mononuclear cell infiltrates at different foci throughout the cortex. Also Glomerular cells damage and tubular cells appear haemorrhagic, hyalinizing, basophilic and regenerating.

In standard drug 400 mg/kg treated group (group 5), the Renal tubular cells showed varying degrees of dilatation with hyaline cast formation in the lumen and the lining tubular epithelial cells showed varying levels of vacuolar degeneration and necrosis. Areas of tubular degeneration and necrosis were observed at a few foci in the cortex along with varying degrees of regenerative changes. The results of histopathological examination of the Kidney revealed significant changes between ajitagada 200mg/kg and standard drug 400mg/kg. Ajitagada 400mg/kg also produced significant histological changes. Ajitagada 200mg/kg produced more regenerative changes in the kidney.

Summary

In experimental study 'The renal protective effect of Ajitagada' was conducted at Veterinary college & sciences, Mannuthi,Thrissur. The present study was undertaken to assess the nephroprotective effect of Ajitagada against gentamicin induced nephrotoxicity in Sprague-dawley rats.Eight adult female Sprague-dawley rats weighing 160-300g,divided into 5 groups comprising eight animals in each group, were used for the study. The experiment was conducted for a period of 30 days for observation although research design period was 15 days. Group 1 served as healthy control group. Gentamicin sulphate was administered to group 2, 3, 4 and 5 at a dose rate of 80mg/kg i.p. for eight days. Group 2 was retained as such without any treatment but administered with 2% Gum-acacia till the completion of experiment. Group3 and 4 were administered with Ajitagada at a dose rate of 200mg/kg and 400mg/kg respectively for 22 days following gentamicin administration. Group 5 was administered with aqueous extract of Ideal drug at a dose rate of 400mg/kg for 22 days for 9^{th} to 30^{th} day following gentamicin administration for eight days.The blood was collected from all the animals on 0^{th}, 9^{th}, 15^{th} and 30^{th} day and serum was used for the estimation of creatinine, urea, albumin and total protein. On 30^{th} day, all the animals were sacrified and kidney was used for the histopathological studies.Serum creatinine level is used as an index of nephrotoxicity. Serum creatinine level which was elevated following gentamicin administration was lowered on 15^{th} day itself by Ajitagada, aqueous extract of ideal drug and without drug. There is no significant change between groups. Higher value of Creatinine naturally recovered to normal value. Serum urea levels were significantly lowered on 15^{th} day by the Ajitagada and the mean values indicated that significant reduction was found with at 200mg/kg and aqueous extract of ideal drug at 400mg/kg.

The serum albumin and total protein showed no significant reduction and they were found to be within normal range throughout the experiment.The

results substantiated by histopathological studies confirmed that treatment with Ajitagada and ideal drug alleviated the gentamicin induced proximal tubular necrosis, interstitial oedema, perivascular oedema and mononuclear cells infiltration. The regenerative changes were predominant with Ajitagada at rate of 200mg/kg.In the present study, Ajitagada and ideal drug showed a striking nephroprotective action in a dose-dependent manner by decreasing the urea levels and lessened the negative effects of Gentamicin induced Nephrotoxicity possibly, by inhibiting free radical mediated process. The diuretic property of Ajitagada may also be one of the contributing factors for the nephroprotection than treatment with the Boerhavia diffusa.From the experiment, it is concluded that Ajitagada possess nephroprotective action and hence this drug can be recommended for the treatment of various Nephrotoxicosis in animals and man.

DISCUSSION

Nephrotoxicity occurs as a disturbance in renal function due tovarious drug Interaction, snake poisoning, inadequate elimination of radioactive contrast materials and chemicals. It is of great concern in patients with renal failure. Nephrotoxicity may limit the clinical usefulness of many diagnostics and therapeutic agents; recognition of factors associated with higher risk for renal injury is of great importance. However the end point of nephrotoxicity is always cell death; therefore it is important to identify the mechanism in addition to the site of action, in order to formulate a strategy for damage prevention. The strategies aimed at ameliorating the nephrotoxicity are of clinical interest. A standard drug which provides nephroprotection is still a major question yet to be answered. Many herbal drugs with action on the urinary system are widely used in human. These herbal drugs are used traditionally but their efficacy and beneficial effects are not scientifically documented. Hence the present study was undertaken to assess the nephroprotective effect of Ajitagada in gentamicin –induced nephrotoxicity.

Serum parameters: Serum creatinine and serum urea (serum markers of kidney function) have been consider the most important manifestations of severe tubular necrosis of kidney. (Ali et al.,2001, Afjal et al.,2004).

Effect on creatinine: Serum creatinine levels were significantly increased in gentamicin group. These results are in accordance with the findings of Ramsammy et al. (1989) that the Serum creatinine Concentration was elevated significantly in gentamicin nephrotoxicity. Elevation of Serum creatinine was marked on 9^{th} and 15^{th} day of the experiment in gentamicin group. The Ajitagada and Boerhavia deffusa showed a reduction in Serum creatinine level on 15^{th} day and by the end of experiment, the Serum creatinine levels of all the treatment groups were comparable with that of normal group. The studies conducted by Shirwaikar et al., (2004) proved that Aerva lanata provided nephroprotection indicated by reduction in Serum creatinine Level which was elevated by gentamicin administration. Kotin's et al., (2004) reported that Hemidesmus indicus, a herbal drug, ameliorated the increased Serum creatinine level thereby providing nephroprotection. Similar results are also

shown by the Ajitagada under study where this herbal Formulation caused a reduction in the creatinine values thereby providing a striking nephroprotective effect.The significant tested between the groups respectively revealed that all the groups were equally effective (statistically insignificant). Thus it is concluded that the Ajitagada at the dose rate of 200mg/kg and 400mg/kg not more effective in providing nephroprotection.

Effect on urea: Gentamicin administration caused an elevation in the level of serum urea.This is in accordance with the findings of Kozat et al. (2007) where elevated serum urea levels were found in gentamicin group. By the end of the experiment, the serum urea levels were significantly reduced in the treatment groups when compared with the control group.Maximum reduction in serum urea levels were shown by Ajitagada at a dose rate of 200mg/kg.Ali et al. (2005) observed that elevated serum urea level was decreades by curcumin at a dose rate of 200mg/kg in gentamicin induced renal damage. The results of the present study are also in accordance with the above findings and the Ajitagada showed significant reduction in serum urea levels thereby contributing towards the nephroprotective effect.The Insignificant tested between the Ajitagada and ideal drug treated group at 200, 400 mg/kg and 400 mg/kg respectively revealed that both the treatment groups were unequally effective (p=0.08).Ajitagada is more effective in compare to ideal drug. But, the Ajitagada produced the significant reduction in serum urea value at the lower dose. Thus it is concluded that the Ajitagada at the dose rate of 200mg/kg is more effective in providing nephroprotection.

Effect on albumin and total protein-

The results of serum parameters like albumin and total Protein revealed no Significant variation. All these parameters were within the normal range (Hrapkiewicz et al., 1998).The study conducted by Kotnis et al., (2004) to assess the nephroprotective effect of Hemidesmus indicus in gentamicin induced renal toxicity in rats revealed no difference in the levels of serum albumin and total protein as compared with the gentamicin and normal control groups.These findings are in accordance with the present study that no significant variation was observed in serum albumin and total protein and gentamicin induced nephrotoxicity had no influence on these parameters.

Histopathological examination of kidney

Histologically, gentamicin group showed severe proximal tubular necrosis and loss of lining epithelium. Besides, there were mononuclear cell infiltrations. In Ajitagada 200mg/kg treated group, varying degrees of regeneration and only small foci of mononuclear infiltration could be seen. Studies conducted by Vardi et al. (2005) found that gentamicin administration showed marked tubular necrosis and desquamation of the cortical epithelial cells and these changes were ameliorated by caffiec acid phenethyl ester.Gentamicin nephrotoxicity occurs as a result of binding of the cationic aminoglycosides with anionic phosphatidyl inositol which are present in the kidney. Recently, it is proposed that aminoglycoside nephrotoxicity results from endocytic retrieval of the drug by megalin, an endocytic-mediated receptor,

present in the apical membrane of proximal convoluted tubules. However, the generation of reactive oxygen species (ROS) has been one of the major contributing factors towardsnephrotoxicity. The mechanism of action through which these plant extracts provide nephroprotection is not clearly understood, but may be due to the scavenging of the free radicals that were generated by gentamicin administration mediated by the action of phytoconstituents present in the plant extracts.the more nephroprotective effect of Ajitagada may be attributed to the excretion of toxic substances alongwith increases diuresis. Further investigations are required to elucidate the exact mechanism underlying this possible beneficial effect.Thus, the findings of the present study validate the nephroprotective effect of Ajitagada for the management of renal disorders.

CONCLUSION

The present study was undertaken to assess the nephroprotective effect of Ajitagada against Gentamicin induced Nephrotoxicity in Sprague-Dawley rats.

The results substantiated by histopathological studies confirmed that treatment with Ajitagada and ideal drug alleviated the Gentamicin induced proximal tubular necrosis, interstitial oedema, perivascular oedema and mononuclear cells infiltration. The regenerative changes were predominant with Ajitagada at rate of 200mg/kg.

In the present study, Ajitagada showed a striking nephroprotective action in a dose-dependent manner by decreasing the serum urea levels, and lessened the negative effects of Gentamicin induced Nephrotoxicity possibly, by inhibiting free radical mediated process. The diuretic property of Ajitagada may also be one of the contributing factors for the nephroprotection.

From the experiment, it is concluded that Ajitagada possess nephroprotective action and hence this drug can be recommended for the treatment of various Nephrotoxicosis in animals and man.

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