Original article

Renoprotective action of the ethanolic extracts of *andrographis paniculata* leaves in albino rats

Dr. Bijoy Bakal , Prof.(Dr)Meghali Chaliha

Department of Pharmacology, Jorhat Medical College, Jorhat, Assam , PIN-785001

Name of the Institute/college: Jorhat Medical College

Corresponding author: Dr. Bijoy Bakal

ABSTRACT:

Background: Over the past few years the use of indigenous drugs has been on the rise along with the modern system of medicine due to its lesser known side effects. Due to wide prevalence of nephropathy, the present study has been undertaken to evaluate the renal protective action of leaves of Andrographis paniculata in Gentamycin induced nephropathy in albino rats.

Aims & Objectives: To study the renal protective effect of Andrographis Paniculata leaves and to compare the effect of Andrographis Paniculata to a standard drug—N-acetylcysteine

Materials and Methods: The present study was conducted in the Department of Pharmacology, Jorhat Medical College, afterethicalclearance. Adult Wistar strain healthy albino rats of either sex, weighing 150-200 g were used. Total animals divided into six groups, each consisting of six animals.Gentamicin (80mg/kg) injected i.p for 8 consecutive days causes mark nephrotoxicity. Nephropathy was confirmed by increase Serum creatinine, blood urea levels and urine output.The drug was administered after induction of nephropathy in different doses.The animals were kept overnight fasted before collection of blood samples. All the animals were kept in metabolic cages for proper collection of urine samples.

Results: The statistical significance between the groups was analyzed using One–way ANOVA, followed by Bonferroni's multiple comparison test. 'p' values of <0.05 were considered as significant. All the extract treated groups with their respective doses showed serum creatinine urea and urine volume-lowering activity ($p<0.05$) at a dose of 200mg/kg b.w.

Conclusion: The ethanolic extract of leaves of A.paniculata at a dose of 200 mg/kg b.w. orally exhibited significant renoprotective ($p<0.05$) activity as evidenced by the decreased serum creatinine, serum urea and decreased urine volume in rats on the 11th day of the experiment.

Key Words: Reno-protective, Andrographis paniculata, albino rats, nephroprotective, nephropathy

INTRODUCTION:

Nephropathy is one of the most common problem of world population. End stage renal disease (ESRD) due to diabetic nephropathy eventually lead to renal failure.[1] Between 20% to 30% of patients with Type 1 or Type 2 diabetes mellitus will have signs or symptoms of diabetic nephropathy during their lifetime.[2]Nephropathy is characterized by presence of persistent proteinuria, hypertension and progressive decline in renal function.[3]Over the past few years the use of indigenous drugs has been on the rise along with the modern system of medicine due to its lesser known side effects. Traditional medicine like Andrographis paniculata (Chirata) leaves were used for renal protective action. It is widely cultivated in southern Asia. Mostly the leaves and

roots have been traditionally used over the centuries for different medicinal purposes in Asia and Europe as a folklore remedy for a wide spectrum of ailments.[4]Keeping in view the present orientation towards indigenous drug development and wide prevalence of nephropathy, the present study has been undertaken to evaluate the purified extract of the plant and its active compound for reno-protective effects in Gentamicin induced nephropathy in albino rats.

AIMS & OBJECTIVE:

The present study is aimed at evaluating the renal protective action of the **ethanolic extract of Andrographis paniculata leaves (EEAPL)** in the albino rats of Wistar strain.The study has been undertaken with a view to explore the renal protective action of an indigenous plant which will be less expensive, less toxic and commonly available.

MATERIALS AND METHODS:

The present study was conducted in the Department of Pharmacology, Jorhat Medical College, Jorhat after due clearance from Institutional Animal ethics committee.

1. Drugs used in the study:

a) Ethanolic Extract of Andrographis paniculata Leaves (EEAPL)

b) Gentamycin

c) N-Acetylcysteine

2. The experimental animals used for the study:

Study was carried out on healthy Albino rats (Rattus norvegicus) of Wistar strain of either sex with a Body weight of 100-200 gms each. Standard diet in sufficient quantity and water given ad libitum to the animals was given during the entire period of the experiment. All the animals were taken care to prevent coprophagy, under ethical consideration.

3. Method of ethanolic extraction of Andrographis paniculata:

The required amount of A.paniculata were collected from local market and identified by a qualified botanist. Leaves of A.paniculata were washed thoroughly with cold water and shade dried in the laboratory at room temperature. The dried leaves were then crushed and powdered in an electrical mixture-grinder, which yielded and amount of 900gms of dry powder and was kept in an air-tight container. 50 grams of powdered leaves was put in Soxhlet apparatus and enough amount of alcohol is put in the round bottom flask. The heating mantle was started at medium heat and the ethanol vapour slowly rises to circulate through the Soxhlet apparatus and the drug extract gets collected at the lower round bottom flask. The process was allowed to proceed slowly until the drug was exhausted with approximately 16 cycles. The ethanol was evaporated in a hot water bath at around 60^0C and a greenish, gummy extract was obtained. The yield at the end of extraction was 4 grams (8% of the dry powder). This procedure was repeated with until the required amount of extract (40 grams) was obtained. The extract was collected in glass Petri dishes, kept in a vacuum desiccator and was used in the experiment.

4. Phytochemical screening of EEAPL:

EEAPL was subjected to qualitative phytochemical analysis for alkaloids, flavonoids, tannins, saponins, terpenoids and phenols as per the standard methods.

5. Acute Oral Toxicity Test:

Acute oral toxicity test for the ethanolic extracts of **Andrographis paniculata leaves.** (EEAPL)was carried out as per OECD Guidelines 425. No sign of toxicity and mortality was recorded among the rats for EEAPL at the dose of 2000mg/kg. Hence, doses

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of 50/kg, 100mg/kg and 200mg/kg were selected arbitrarily for study.

6. Preparation of suspension of ethanolic extract of A.paniculata:

The ethanolic extract of A.paniculata (EEAPL), thus obtained was mixed with 3% (3mg in 100ml of distilled water) aqueous suspension of gum acacia. The doses used are 50mg/kg, 100mg/kg and 200mg/kg bodyweight for the respective group.

7. Preparation of N-acetylcysteine suspension:

The stock solution was prepared by dissolving 10mg of N-acetylcysteine in 5ml of aqueous suspension of 3% gum acacia.

8. Induction of Renal Injury:

Gentamicin was used as a nephrotoxic to cause renal injury. Gentamicin (80mg/kg) injected i.p for 8 consecutive days causes mark nephrotoxicity. [5]

9. Method of Renoprotective Study in Albino Rats (Wistar strain):

The method of Pratibha et al., with slight modification was followed to investigate the Renoprotective effect of EEAPL. The experiment was carried out for a period of 10 days, after induction of nephrotoxicity. For this purpose, 36 healthy albino rats (Wistar strain) of either sex were collected from the Central Animal House of Jorhat Medical College, Jorhat. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week and they were provided with standard diet and water in sufficient quantity, as per the recommendations of CPCSEA for laboratory animal facilities. For the experiment, the animals were weighed, recorded, numbered and randomly divided into six groups of 6 animals each.

Six rats were kept as normal control group without inducing nephropathy. Rest 30 rats were induced nephropathy by Gentamicin (80mg/kg) injected i.p for 8 consecutive days causing mark nephrotoxicity. Nephropathy was confirmed by increase Serum creatinine, blood urea levels and urine output,. The animals were kept overnight fasted before collection of blood samples. All the animals were kept in metabolic cages for proper collection of urine samples. The induction of nephropathy was considered as Day 1 of treatment. The animals were grouped as follows:

10. Grouping and treatment schedule:

Renal Protective Model: Total animals will be divided into six groups, each consisting of six animals.

Group I : Control group on Standard Diet (SD)

Group II: Albino rats with Gentamicin induced nephropathy + SD

Group III :Albino rats with Gentamicin induced nephropathy + SD + N-acetylcysteine

Group IV: Albino rats with Gentamicin induced nephropathy + SD + EEAPL 50 mg/kg body weight

Group V : Albino rats with Gentamicin induced nephropathy + SD + EEAPL 100 mg/kg body weight

Group VI: Albino rats with Gentamicin induced nephropathy + SD + EEAPL 200 mg/kg body weight

All the animals used for the experiment were kept under observation for daily food and water intake. The drugs were administered orally to the animals as per the doses, for a period of 10 days, by means of a feeding tube. After the last application, rats were placed in metabolic cages for 24 hours of urine collection to determine the urine output.

11. Method of Blood Collection:

At the end of 24 hours, the rats were anaesthetized and under all aseptic and antiseptic measure, blood samples were collected from the orbital sinus with the help of a capillary tube by pressing the thumb

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behind the angle of the jaw resulting in engorgement of the retro-orbital plexus. The blood was collected in plain plastic tubes, left to stand at 48°C for 1 hour. The serum obtained was stored at 58°C until analysis.

12. Method of Serum Creatinine Estimation:

Serum creatinine was estimated using a creatinine kit by using Modified Jaffe's Kinetic Method.

13. Serum Creatinine was estimated from the following formula:

Creatinine in mg/dl = $\frac{\Delta AT}{\Delta AS}$ x 2.0

Thus this procedure gave the measurement of serum creatinine of particular sample. This procedure was repeated for each blood sample.

14.Method of Serum Urea Estimation:

Serum Urea was estimated by Berthelot method using an Urea kit. At the end of incubation the absorbance of the Standard (AS), Unknown (AU), was measured against blank at 578 nm.

15. Method of Urine Volume Estimation:

24 hours urine collection was done from the container below the metabolic cage with proper care to prevent the mixing of faeces with the urine sample. The samples were measured in a cylindrical jar and the urine volume was determined.

OBSERVATION AND RESULTS:

The statistical significance between the groups was analyzed separately using One–way Analysis of Variance (ANOVA), followed by Bonferroni's multiple comparison test and paired t-test wherever required. The significance was expressed by 'p' values, as mentioned in the tables. 'p' values of <0.05 were considered as significant. The statistical analysis was carried out using GraphPad prism 7 software.

1. Phytochemical screening of EEAPL:

The phytochemicals revealed during the qualitative phytochemical analysis of EEAPL are depicted in Table 1. The extract was positive for predominantly deterpenoids, lactones and flavonoids.

Table 1 : Phytochemicals in EEAPL

PHYTOCHEMICALS	EEAPL
Deterpenoids	Present
Lactones	Present
Flavonoids	Present
Tannins	Present
Phenolic compounds	Present
Saponins	Present

2. Effect of EEAPL on serum creatinine level in nephrotoxic rats:

The effect of EEAPL on serum creatinine in gentamicin induced nephrotoxic albino rats is given in Table–2. In Normal control group, no significant change in creatinine level was observed during the study period. Moreover there was no significant ($p>0.05$) difference of the baseline creatinine levels among the various groups.There was significant rise ($p<0.05$) in creatinine level in nephrotoxic rats, after 8 days of i.p. gentamicin injection, compared to normal control group. This day was recorded as the

1st day of treatment.

On 11th day of treatment, the creatinine was measured again. In the gentamicin control group there was further increase in the serum creatinine level on the 11th day, which was also significant ($p<0.05$).In the standard (NAC) group and the extract treated groups, the serum creatinine levels showed a decreasing trend. The standard group and EEAPL 200mg/kg b.w. showed significant ($p<0.05$) difference in comparison to the gentamicin control group at the end of 10 days of treatment i.e. on the 11th day. The percentage reductions of serum creatinine on day 11 when compared to the gentamicin control were 76.38%, 02.87%, 32.12% and 64.78.57% for groups of renoprotective standard drug, EEAPL 50mg/kg b.w., EEAPL 100mg/kg b.w. and EEAPL 200mg/kg b.w., respectively.All the extract treated groups with their respective doses showed creatinine lowering effect. However serum creatinine-lowering activity of EEAPL 200mg/kg b.w. was found significantly ($p<0.05$) higher than EEAPL 50mg/kg b.w. and EEAPL 100mg/kg b.w. at Day 11.There was no significant difference between the standard renoprotective drug (NAC) and EEAPL 200mg/kg b.w. ($p>0.05$). Hence it can be said that EEAPL 200mg/kg b.w is comparable to the standard drug N-acetylcysteine.

Table2: Effect of EEAPL on mean serum creatinine levels

Mean serum creatinine(in mg/dl)			
Groups	Day 0 (Baseline)	Day 1 (after induction of nephrotoxicity)	Day 11 (at the end of 10th day of treatment)
Group IB (Normal Control)	0.71± 0.06	0.75± 0.04	0.71± 0.07
Group IIB (Gentamicin Control)	0.76± 0.4	9.0± 0.46[a]	9.40± 0.44[a]
Group IIIB (Standard Drug)	0.73± 0.07	7.3± 0.35[a]	2.22± 0.22[a,b]
Group IVB (EEAPL 50mg/kg)	0.075± 0.05	8.0± 0.74[a]	9.13± 0.22[a,c]
Group VB (EEAPL 100mg/kg)	0.66± 0.08	8.2± 0.61[a]	6.38± 0.41[a,b,c]

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Group VIA (EEAPL 200mg/kg)		0.68± 0.07	8.5± 0.57[a]	3.31± 0.30[a,b]
ANOVA	**p**	>0.05	<0.05*	<0.05*

Values are expressed as MEAN±SEM; (n=6). One Way ANOVA followed by Bonferroni test is done. [a] $p<0.05$ when compared to the Normal control group, [b]$p<0.05$ when compared to the Gentamicin control group, group [c]$p<0.05$ whencompared to the Standard drug.* symbolize significant p values.

3. Effect of EEAPL on serum urea level in nephrotoxic rats:

The effect of EEAPL on serum urea in gentamicin induced nephrotoxic albino rats is given in Table–3. In Normal control group, no significant change in serum urea level was observed during the study period.Moreover there was no significant ($p>0.05$) difference of the baseline urea levels among the various groups.

There was significant rise ($p<0.05$) in urea level in nephrotoxic rats, after 8 days of i.p. gentamicin injection, compared to the normal control group. This day was recorded as the 1st day of treatment.On 11th day, the urea level was measured again. In the gentamicin control group there was further increase in the urea level on the 11th day, which was also significant ($p<0.05$).In the standard (NAC) group and extract treated groups, the serum urea levels showed a decreasing trend. The standard group and EEAPL 200mg/kg b.w. showed significant ($p<0.05$) difference in comparison to the gentamicin control group at the end of 10 days of treatment i.e. on the 11th day. The percentage reductions of serum urea on day 11 when compared to the gentamicin control were 56.24%, 34.9%, 42.49% and 52.58% for groups of renoprotective standard, EEAPL 50mg/kg b.w., EEAPL 100mg/kg b.w. and EEAPL 200mg/kg b.w., respectively. All the extract treated groups with their respective doses showed serum urea lowering effect. However serum urea-lowering activity of EEAPL 200mg/kg b.w. was found significantly ($p<0.05$) higher than EEAPL 50mg/kg bw and EEAPL 100mg/kg b.w. at Day 11.There was no significant difference between the standard renoprotective drug (NAC) and EEAPL 200mg/kg b.w. ($p>0.05$). Hence it can be said that EEAPL 200mg/kg b.w is comparable to the standard drug N-acetylcysteine.

Table 3. Effect of EEAPL on serum urea level

Mean serum urea(in mg/dl)				
Groups		Day 0 (Baseline)	Day 1 (after induction of nephrotoxicity)	Day 11 (end of 10th day of treatment)
Group IB (Normal Control)		24.0± 1.17	24.8± 1.04	24.2± 1.31
Group IIB (Gentamicin Control)		23.2± 0.68	67.1± 9.05[a]	79.3± 2.95 [a]
Group IIIB (Standard Drug)		23.6± 0.61	73.6± 3.26 [a]	34.7± 1.24 [a,b]
Group IVB (EEAPL 50mg/kg)		22.7± 0.59	62.7± 7.75 [a]	51.6± 1.30 [a,b,c]
Group VB (EEAPL 100mg/kg)		23.1± 1.20	74.9± 2.69 [a]	45.6± 1.20 [a,b,c]
Group VIA (EEAPL 200mg/kg)		25.0± 1.05	76.3± 1.58 [a]	37.6± 0.61 [a,b]
ANOVA	**p**	>0.05	<0.05	<0.05

Values are expressed as MEAN±SEM; (n=6). One Way ANOVA followed by Bonferroni test is done. [a]p<0.05 when compared to the Normal control group [b]p<0.05 when compared to the Gentamicin control group, [c]p<0.05 when compared to the Standard drug.

4. Effect of EEAPL on 24 hour urine volume in nephrotoxic rats:

The effect of EEAPL on urine volume in gentamicin induced nephrotoxic albino rats is given in Table–4.In Normal control group, no significant change in 24 hour urine volume was observed during the study period. Moreover there was no significant (p>0.05) difference of the baseline urine volume among the various groups.There was significant rise (p<0.05) in the urine volume in nephrotoxic rats, after 8 days of i.p. gentamicin injection, compared to the normal control group. This day was recorded as the 1st day of treatment.

On 11th day, in the gentamicin control group there was further increase in the 24 hour urine volume, which was also significant (p<0.05).In the standard (NAC) group and extract treated groups the 24 hour urine volume showed a decreasing trend. The standard group and EEAPL 200mg/kg b.w. showed significant (p<0.05) difference in comparison to the gentamicin control group at the end of 10 days of treatment i.e. on the 11th day. The percentage reductions of urine volume on day 11 when

compared to the gentamicin control were 57.59%, 30.90%, 42.27% and 50.45% for groups of renoprotective standard, EEAPL 50mg/kg b.w., EEAPL 100mg/kg b.w. and EEAPL 200mg/kg b.w., respectively.

All the extract treated groups with their respective doses showed urine volume lowering effect, suggesting improvement of renal function. However urine volume lowering effect of EEAPL 200mg/kg b.w. was found significantly ($p<0.05$) higher than EEAPL 50mg/kg b.w. at Day 11.There was no significant difference between the standard renoprotective drug (NAC) and EEAPL 200mg/kg b.w. ($p>0.05$). Hence it can be said that EEAPL at a dose of 200mg/kg b.w is comparable to the standard drug N-acetylcysteine.

Table 4. Effect of EEAPL on 24-hour urine volume

Mean 24 hour urine volume (in ml)				
Groups		Day 0 (Baseline)	Day 1 (After induction of nephrotoxicity)	Day 11 (end of 10th day of treatment)
Group IB (Normal Control)		8.41± 0.24	7.83± 0.21	7.83± 0.21
Group IIB (Gentamicin Control)		8.67± 0.36	18.4± 0.65[a]	22.0± 1.05[a]
Group IIIB (Standard Drug)		9.17± 0.28	18.3± 0.34[a]	9.33± 0.40[a,b]
Group IVB (EEAPL 50mg/kg)		9.0± 0.37	18.7± 1.86[a]	15.2± 0.49[a,b,c]
Group VB (EEAPL 100mg/kg)		8.41± 0.40	18.8± 0.84[a]	12.7± 0.65[a,b,c]
Group VIA (EEAPL 200mg/kg)		8.91± 0.35	18.5± 0.53[a]	10.9± 0.40[a,b]
ANOVA	**p**	>0.05	<0.05	<0.05

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Values are expressed as MEAN±SEM; (n=6). One Way ANOVA followed by Bonferroni test is done. [a]p<0.05 when compared to the Normal control group [b]p<0.05 when compared to the Gentamicin control group, [c]p<0.05 when compared to the Standard drug.

DISCUSSION:

The renoprotective effect of the plant extract in nephrotoxic rats had been evaluated by the method described by Pratibha et al. (2009) with some modifications.Wistar albino rats were selected for renoprotective action. Gentamicin (80mg/kg/day) is given daily for 8 days through i.p. route. for induction of nephrotoxicity.[5]The typical clinical manifestation of gentamicin toxicity is nonoliguric or even polyuric renal excretion dysfunction accompanied by an increase in plasma creatinine, urea and other metabolic products.[6]

In our study, there was significant (p<0.05) decrease in Serum creatinine in the EEAPL treated group when compared to Gentamicin control group. The standard drug (NAC), also showed significant (p<0.05) lowering of serum creatinine in the nephrotoxic group, after treatment of 10 days, when compared to Gentamicin control group. Creatinine concentrations in blood was significantly (p<0.05) increased in the Gentamicin control group when compared with the normal control group indicating the induction of severe nephrotoxicity. In addition to that Padmalochana et al. (2015) demonstrated similar effect of creatinine lowering action in ethanolic, aqueous, acetone extract of A.paniculata and Cystone as standard drug.[7]

In our study serum urea concentrations was significantly (p<0.05) increasedin the Gentamicin control group when compared with the normal control group indicating the induction of severe nephrotoxicity.The standard drug (NAC), showed significant (p<0.05) lowering of serum urea in the nephrotoxic group, after treatment of 10 days, when compared to Gentamicin control group. Moreover there was also significant (p<0.05) decrease in serum urea in the EEAPL 50mg/kg b.w., 100mg/kg b.w. and 200 mg/kg b.w. treated group when compared to Gentamicin control group. The EEAPL 200mg/kg b.w. has most potent serum urea lowering action when compared to the other dosage of EEAPL, which is comparable to the standard drug.Nalamolu et al. (2006) reported to have serum urea lowering property of Andrographis paniculata root extract in experimental animals.[8]

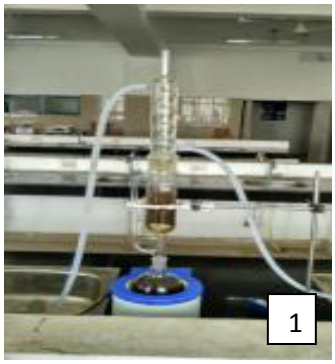
Gentamicin induced polyuria and a defective urine concentration mechanism on day 7 of i.p. injection, suggesting gentamicin-induced collecting duct cell dysfunction as reported by Ahmed et al.(2014).[9] In our study the nephrotoxic rats exhibited polyuria, which improved on treatment with the EEAPL extract and standard drug. EEAPL in a dose of 200mg/kg b.w. is comparable to the standard drug in lowering the urine volume.

The probable mechanism of Renoprotection of EEAPL is due to the presence of phytochemical like deterpenoids, flavonoids, tannins, saponins and phenols present in our extract.

CONCLUSION:

Renoprotective effects may be attributed to the phytochemicals like diterpenoids present in the extract of Andrographis paniculata which have antioxidant property. Thus, it can be concluded that the EEAPL holds enormous potential for the development of drugs for the prevention and control of diabetes with additional renoprotective property. However, there is need for further elaborate studies of quantitative estimation of phytochemicals. Pharmacokinetic studies in terms of bioavailability

and pharmacodynamics study for evaluation of safety and efficacy of the active ingredients on large experimental animals and human beings which can provide more definitive data regarding its therapeutic potential and exact mechanism of action for its better economic and therapeutic utilization is also needed in near future.



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