



Available Online through
www.ijptonline.com

NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *BACCAUREA RAMIFLORA* AND *MICROCOS PANICULATA* AGAINST GENTAMYCIN AND PARACETAMOL INDUCED NEPHROTOXICITY IN ALBINO RATS

Suwendu Saha* T. Shivaraj Gouda¹ S. Vijaya Srinivas²

*Department of Pharmacology, Malla Reddy Institute of Pharmaceutical Sciences, Maisammaguda, Dhulapally, Secunderabad-500100, Telengana, India.

¹Department of Pharmacology, NET Pharmacy College, Raichur, India.

²Department of Pharmaceutics, Prasad Institute of Pharmaceutical Sciences, Jangaon, Telengana, India.

Email: suwendu.belonia@gmail.com

Received on: 10-12-2017

Accepted on: 19-01-2018

Abstract

The present study was undertaken to investigate the nephro protective effect of ethanolic leaf extract of *Baccaurea ramiflora* and *Microcos paniculata* against gentamycin and paracetamol induced nephrotoxicity in rats. The study is carried out by using seven groups of rats. N-acetyl cystone and silymarin were taken as standard drugs. The parameters estimated are RBC content, haemoglobin content, urea and creatinine levels. The extracts showed nephro-protective activity by significantly reducing the levels of blood urea, serum creatinine, increasing the red blood cell count and haemoglobin content. Histopathological studies were also confirmed the nephroprotective action of ethanolic leaf extract of *Baccaurea ramiflora* and *Microcos paniculata*. Present investigation revealed that *Baccaurea ramiflora* and *Microcos paniculata* showed nephroprotective effect on gentamycin and paracetamol induced nephrotoxicity in rats which may be due to the presence of flavonoids and related compounds.

Keywords: *Baccaurea ramiflora*, *Microcos paniculata*, Paracetamol, Gentamycin, N-acetyl cystone, Creatinine, Nephrotoxicity, Nephroprotective activity.

Introduction: The kidneys are the final common pathway for excretion of many drugs and their metabolites, and thus are often subjected to high concentrations of potentially toxic substances. Consequently, direct toxic damages

occur, usually affecting renal tubular cells and renal papillae. Many classes of drugs can cause kidney damages.¹ The kidney also ensures the excretion of metabolic waste and exogenous products, while maintaining the essential elements to the body.² Drugs are a common source of acute nephrotoxicity. Drug toxicity³ remains an important cause of acute kidney injury that in many circumstances can be prevented or at least minimized by vigilance and early intervention. Nephrotoxicity is one of the most common kidney problems and occurs when body is exposed to a drug or toxin.⁴ The rate of drug-induced nephrotoxicity has been increasing with the ever increasing number of drugs and easy availability of over-the-counter medication viz. nonsteroidal anti-inflammatory drugs (NSAIDs). Angiotensin converting enzyme inhibitors, antibiotics, NSAIDs, and contrast agents are the major culprit drugs contributory to damage of kidney.⁵ Nephroprotective agents are the substances which possess protective activity against nephrotoxicity. Medicinal plants have curative properties due to the presence of various phytoconstituents. Earlier literatures have prescribed various herbs for the cure of renal disorders. Co-administration of several medicinal plants possessing the nephroprotective activity along with different nephrotoxic agents which may attenuate its toxicity.⁶ Gentamycin is a broad spectrum antibiotic⁷ frequently used due to its high efficacy against gram negative bacteria. The principal side effect of this class of antibiotics is nephrotoxicity. Its use is restricted due to the development of ototoxicity and nephrotoxicity. Paracetamol (acetaminophen) is a widely used antipyretic and analgesic which produces acute kidney damage if overdoses are consumed. Paracetamol is mainly metabolized in liver to excretable glucuronide and sulphate conjugates.^{8,9}

***Baccaurea ramiflora* (family: Euphorbiaceae)** is native to Southeast Asia region and is found distributed in the sub-Himalayan tract, mainly from Nepal to Sikkim, Darjeeling hills, Arunachal Pradesh, Tripura, Assam, Bhutan, Burma, Penninsular Malaysia, Tibet and Andaman islands. It is an evergreen tree reaching a height of about 5-10 m. The leaf is simple, alternately arranged, with petiole.¹⁰ ***Microcos paniculata* (family: Euphorbiaceae)** is a shrub that is abundant in secondary forests and also grown as hedges. *Microcos* is tall semi-deciduous tree, sometimes shrubby. Leaves 10-15 cm long, elliptic-oblong, acuminate, entire or slightly and irregularly toothed.^{11,12} Many studies have established that *Baccaurea ramiflora* and *Microcos paniculata* leaves extract have potent anti-inflammatory, ulcer protective, and hepatoprotective properties. Since no work was reported relating to

nephroprotective effect of *Baccaurea ramiflora* and *Microcos paniculata* plant leaves, the present study was designed to investigate the nephroprotective effect of the ethanolic extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on gentamycin and paracetamol induced nephrotoxicity in albino rats.

Materials and Methods

Collection of plant materials: The leaves of *Baccaurea ramiflora* and *Microcos paniculata* belonging to family Euphorbiaceae were collected from local market of Belonia, Tripura, India during May – July and authenticated (ID No. is BOT/HEB/AC23072011 and BOT/HEB/AC23072512) by Dr. B. K. Datta, Professor of Botany, Plant Taxonomy and Biodiversity Laboratory, Department of Botany, Tripura, India.

Preparation of crude drug extracts

After collection of the plants, the leaves of both the plants were rinsed thoroughly in tap water and dried in shade for about 20 days under controlled temperature (25 ± 2 °C). Then the crude material was powdered, passed through a 40 mesh sieve and stored in a well closed container for further usage.

Coarsely powdered and dried leaves were successively soxhlated using petroleum ether, chloroform, ethanol and water for 72h. The extracts were filtered and the solvents were evaporated to dryness under reduced pressure in a rotary evaporator at 40 °C to 45 °C. A brown residue was recovered from flask with 12% yield of ethanol extract.

Animals

Wistar albino rats of either sex were used for the study of the crude drug extracts. Permission for conduct of these experiments was obtained from Institutional Animal Ethics Committee (IAEC) **Regd. No.** 1662/PO/Re/S/12/CPCSEA.

The rats were acclimatized to the laboratory conditions for a week before the start of the experiments; they were maintained as per the Institutional ethical committee (IAEC) norms. Animals were housed at standard conditions of temperature (27 ± 2 °C), relative humidity 44-56% and 10/14 h light/dark cycle, respectively, for 1 week before and during the experiments.

Animals were provided with standard diet and the food was withdrawn 18h before the start of the experiment and water *add libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and ethical guidelines for the investigation of experimental pain in conscious animals.¹³

Phytochemical studies:

All the extracts were subjected for phytochemical study.¹⁴

Acute toxicity studies

The acute toxicity study for ethanolic extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves were performed using albino rats as per OECD guidelines- 425 (OECD, 2008).¹⁵ The animals were fasted overnight before the experiment and maintained under standard conditions. All the extracts were administered orally in increasing dose and found safe up to dose of 2000mg/kg for all extracts.

Experimental animal and design

Gentamycin induced nephrotoxicity in rats

Gentamycin induced model^{16,17} was used in this study. The rats were systematically divided into seven groups of 6 rats each.

Twelve hours before the experiment began, the rats were fasted of feed but distilled water was made available ad libitum.

Group I served as a control group and received distilled water p.o for 8 days.

Group II served as toxic control, gentamycin to induce nephrosis. The gentamycin group received 80 mg/kg/day by the intraperitoneal (i.p) route daily once for 8 days.

Group III received Silymarin 25mg/kg/day p.o as reference standard once daily for 6 weeks and along with gentamycin during last 8 days.

Group IV rats received 100mg/kg b.w of ethanolic extract of *Baccaurea ramiflora* leaves (EEBR1 100) once daily orally for 6 weeks and along with gentamycin during last 8 days.

Group-V received 200 mg/kg b.w of ethanolic extract of *Baccaurea ramiflora* leaves (EEBR2 200) once daily orally for 6 weeks and along with gentamycin during last 8 days respectively.

Group VI rats received 100mg/kg b.w of ethanolic extract of *Microcos paniculata* leaves (EEMP1 100) once daily orally for 6 weeks and along with gentamycin during last 8 days.

Group-VII received 200 mg/kg b.w of ethanolic extract of *Microcos paniculata* leaves (EEMP2 200) once daily orally for 6 weeks and along with gentamycin during last 8 days respectively.

Paracetamol induced nephrotoxicity in rats:

Paracetamol induced model was used in this study. The rats were systematically divided into seven groups of 6 rats each. Twelve hours before the experiment began, the rats were fasted of feed but distilled water was made available ad libitum.

Group I served as a control group and received distilled water p.o for 8 days.

Group II served as toxic control, paracetamol to induce nephrosis. The paracetamol group received 750 mg/kg/day by the intraperitoneal (i.p) route daily once for 8 days. Group III received N-acetyl cysteine 20mg/kg/day p.o as reference standard once daily for 6 weeks and along with paracetamol during last 8 days.

Group IV rats received 100mg/kg b.w of ethanolic extract of *Baccaurea ramiflora* leaves (EEBR1 100) once daily orally for 6 weeks and along with paracetamol during last 8 days.

Group-V received 200 mg/kg b.w of ethanolic extract of *Baccaurea ramiflora* leaves (EEBR2 200) once daily orally for 6 weeks and along with paracetamol during last 8 days respectively.

Group VI rats received 100mg/kg b.w of ethanolic extract of *Microcos paniculata* leaves (EEMP1 100) once daily orally for 6 weeks and along with paracetamol during last 8 days.

Group-VII received 200 mg/kg b.w of ethanolic extract of *Microcos paniculata* leaves (EEMP2 200) once daily orally for 6 weeks and along with paracetamol during last 8 days respectively.

On the 9th day blood samples were collected via retro orbital puncture in overnight fasted animals under mild ether anesthesia. The collected samples were allowed to clot and serum was separated by centrifuging at 2500 rpm for 15 mins and analyzed for serum creatinine, urea, RBC and haemoglobin by using kits.

Histopathological studies of rat kidneys

Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological evaluation. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope (magnification power-40X).¹⁸

Statistical analysis

The statistical significance was evaluated using the student's t-test. The values are expressed as mean \pm SEM and $p < 0.05$ was considered significant in all comparisons.

Result

Phytochemical study: All the extracts subjected for phytochemical investigation revealed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids.

Acute toxicity studies

The extracts were found to be safe in the dose used did not show any signs and symptoms of toxicity and there was no mortality up to a dose of 2000 mg/kg, b.w. Hence 100 and 200 mg/kg b.w. p.o. were selected for the activity.

Effect of extracts on Creatine, Urea, RBC and Haemoglobin

The results of nephroprotective effect of extracts on gentamycin and paracetamol intoxicated rats are shown in **Table 1, 2**. Values of Table 1 & 2 showed that serum creatinine and serum urea levels were significantly increased in rats treated with only gentamycin and paracetamol whereas RBC and haemoglobin levels were significantly decreased. But treatment with the N-acetyl cystone, Silymarin and ethanolic extracts of leaves of *Baccaurea ramiflora* and *Microcos paniculata* for 6 weeks reversed the effect of gentamycin and paracetamol indicating nephroprotective activity. The plant extract showed dose dependent nephroprotective effect.

Table-1: Nephroprotective Activity of *Baccaurea ramiflora* and *Microcos paniculata* in gentamycin induced albino rats.

Group	Dose	Urea (mg/dl)	Creatinine (mg/dl)	RBC (million cells/mm ³)	Haemoglobin (g/dl)
I	Normal Control	25.46 \pm 1.33	1.81 \pm 0.027	7.73 \pm 0.024	12.72 \pm 0.60
II	Toxic Control Gentamycin (80mg/kg)	56.73 \pm 2.116	3.10 \pm 0.013	5.51 \pm 0.028	7.85 \pm 0.31
III	Silymarin (25mg/kg)	25.64 \pm 2.286**	1.93 \pm 0.04**	7.28 \pm 0.037***	13.86 \pm 0.85***
IV	EEBR1 (100mg/kg)	29.13 \pm 2.480**	2.14 \pm 0.011**	6.28 \pm 0.030***	12.36 \pm 0.94***

V	EEBR2 (200mg/kg)	27.70±2.284**	2.01±0.022**	6.50±0.023***	12.76±0.60***
VI	EEMP1 (100mg/kg)	31.45±2.718**	2.32±0.021**	5.75±0.068***	11.62±0.21***
VII	EEMP2 (200mg/kg)	29.85±3.31**	2.15±0.010**	6.21±0.026***	12.31±0.58***

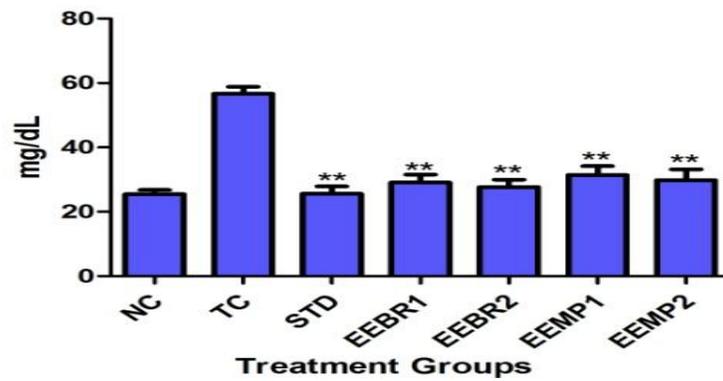
Values are given as Mean ± SEM (n=6 rats). * Significant at P<0.005 when gentamycin treated compared with control group. **Significant at P<0.01 when gentamycin treated compared with control group. ***Significant at P<0.001 when gentamycin treated compared with control group.

Table-2: Nephroprotective Activity of *Baccaurea ramiflora* and *Microcos paniculata* in paracetamol induced albino rats.

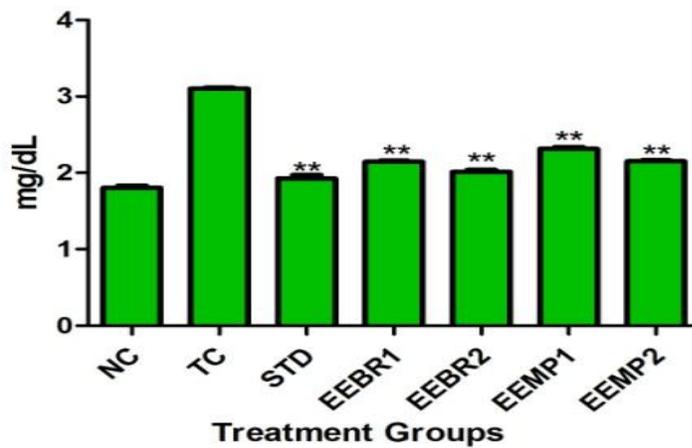
Group	Dose	Urea (mg/dl)	Creatinine (mg/dl)	RBC (million cells/mm ³)	Haemoglobin (g/dl)
I	Normal Control	22.8±1.134	1.65±0.014	8.59±0.029	14.33±0.44
II	Toxic Control Paracetamol (750mg/kg)	51.18±1.77	2.82±0.01	6.12±0.059	8.57±0.27
III	Standard N-acetyl cystone (20mg/kg)	23.4±1.89**	1.78±0.033**	8.13±0.053***	14.71±0.70***
IV	EEBR1 (100mg/kg)	26.88±1.70**	1.93±0.035**	6.98±0.028***	13.40±0.20***
V	EEBR2 (200mg/kg)	25.05±2.26**	1.82±0.017**	7.23±0.067***	14.11±0.82***
VI	EEMP1 (100mg/kg)	28.19±2.18**	2.11±0.058**	6.41±0.061**	12.8±0.53***
VII	EEMP2 (200mg/kg)	26.85±2.33**	1.98±0.028**	6.90±0.056***	13.63±0.55***

Values are given as Mean ± SEM (n=6 rats). * Significant at P<0.005 when paracetamol treated compared with control group. **Significant at P<0.01 when paracetamol treated compared with control group. ***Significant at P<0.001 when paracetamol treated compared with control group

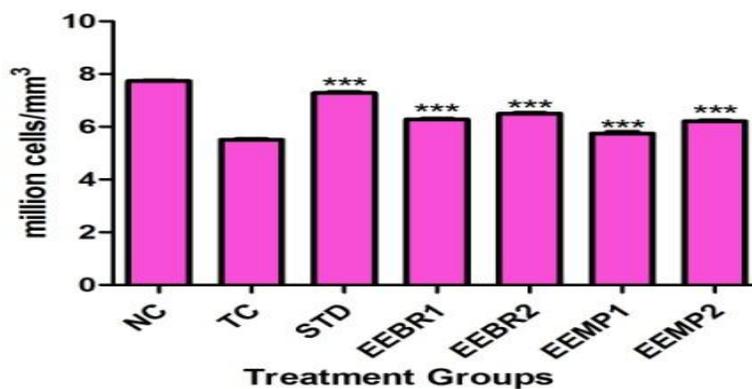
EEBR: Ethanolic extract of *Baccaurea ramiflora*; **EEMP:** Ethanolic extract of *Microcos paniculata*.



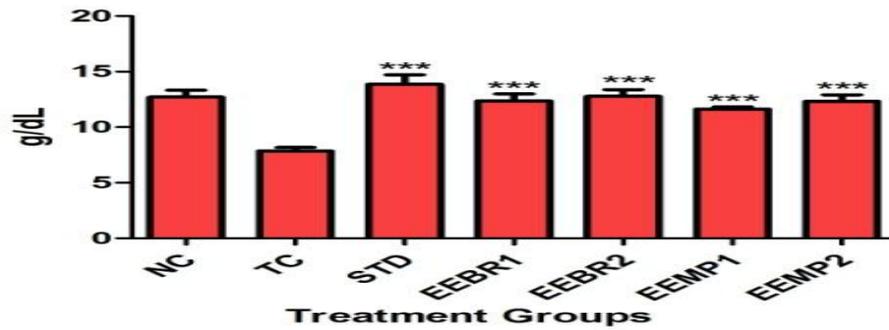
Graph-1. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Urea of gentamycin-induced nephrotoxic albino rats.



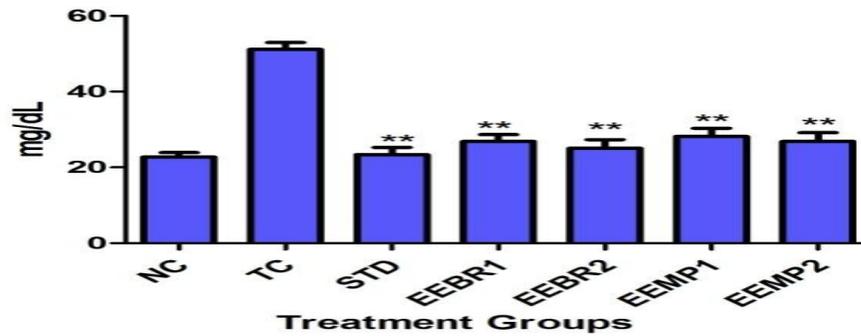
Graph 2. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Creatinine of gentamycin-induced nephrotoxic albino rats.



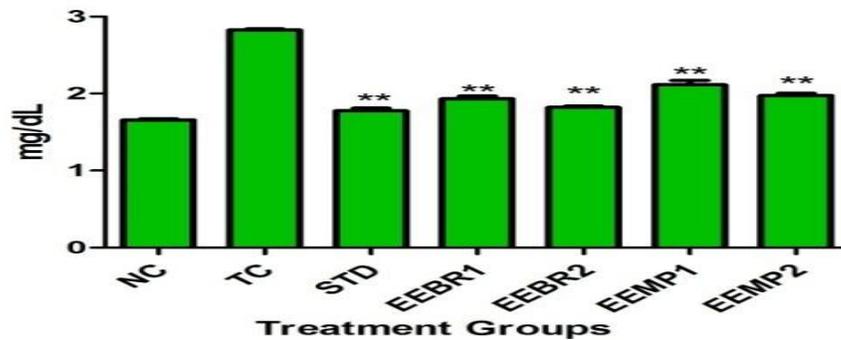
Graph 3. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on RBC of gentamycin-induced nephrotoxic albino rats.



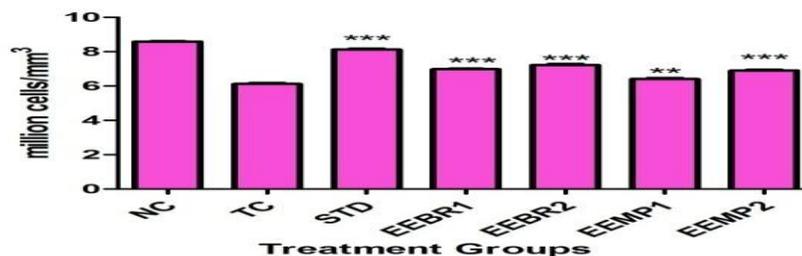
Graph 4. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Haemoglobin of gentamycin-induced nephrotoxic albino rats.



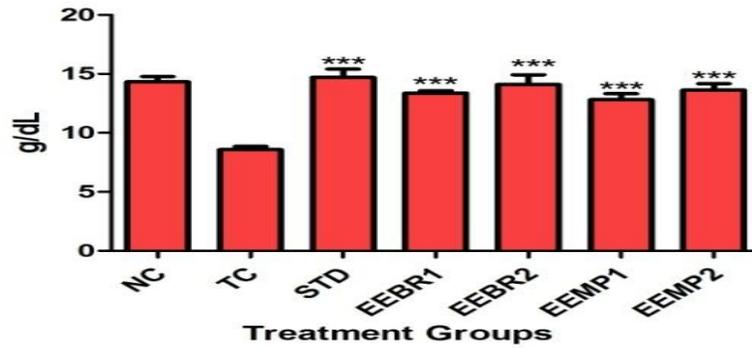
Graph 5. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Urea of paracetamol-induced nephrotoxic albino rats.



Graph 6. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Creatinine of paracetamol-induced nephrotoxic albino rats.



Graph 7. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on RBC of paracetamol-induced nephrotoxic albino rats.



Graph 8. Effect of ethanol extract of *Baccaurea ramiflora* and *Microcos paniculata* leaves on Haemoglobin of paracetamol-induced nephrotoxic albino rats.

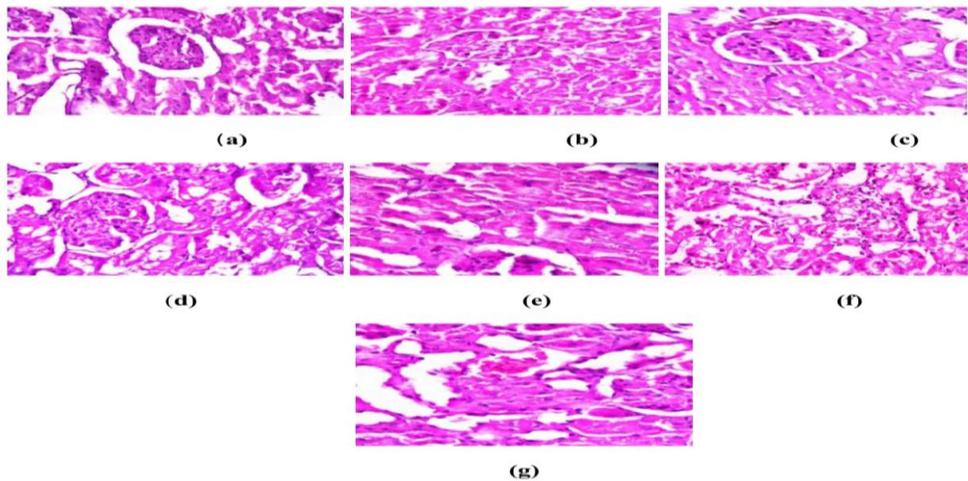


Figure 1: Histopathological changes of kidney in gentamycin induced nephrotoxicity.

(a) Normal control (b) Gentamycin 80mg/kg (c) Silymarin 25mg/kg (d) EEBR 100mg/kg (e) EEBR 200 mg/kg (f) EEMP 100mg/kg (g) EEMP 200mg/kg

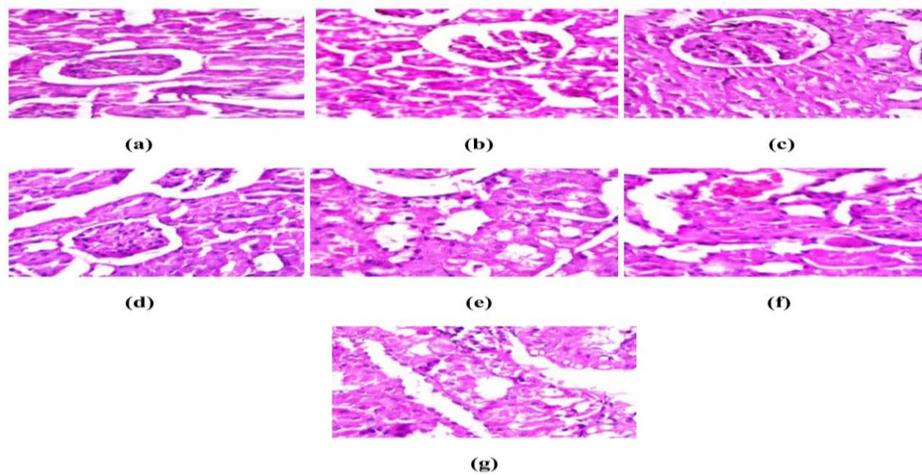


Figure 1: Histopathological changes of kidney in paracetamol induced nephrotoxicity.

(a) Normal control (b) Paracetamol 750 mg/kg (c) N-acetyl cystone 20mg/kg (d) EEBR 100mg/kg (e) EEBR 200 mg/kg (f) EEMP 100mg/kg (g) EEMP 200mg/kg

Table-3: Histopathological observations of kidney in gentamycin-induced nephrotoxicity in rats.

MICROSCOPY	NC	TC	STD	EEBR 1	EEBR 2	EEMP 1	EEMP 2
Cytoplasmic vacuoles	-	+	-	+	+	+	+
Glomerular congestion	-	+	+	+	-	+	+
Interstitial Haemorrhage	-	-	-	-	-	-	-
Interstitial odema	-	+	-	-	-	-	-
Intravascular Haemolyse	-	-	-	-	-	-	-
Loss of Brush Broders	-	+	-	+	+	+	+
Petitubular inflammation	-	+	-	+	-	+	-
Tubular cell swelling	-	+	-	+	-	-	-
Tubular congestion	-	+	+	+	+	+	+
Tubular Degeneration	-	+	-	-	-	+	-
Tubular Desquamation	-	+	-	-	-	+	-
Tubular necrosis	-	+	-	+	-	+	-

Table-4: Histopathological observations of kidney in paracetamol-induced nephrotoxicity in rats

MICROSCOPY	NC	TC	STD	EEBR 1	EEBR 2	EEMP 1	EEMP 2
Cytoplasmic vacuoles	-	+	-	+	+	+	+
Glomerular congestion	-	+	+	+	-	+	+
Interstitial Haemorrhage	-	-	-	-	-	-	-
Interstitial odema	-	+	-	+	-	+	-
Intravascular Haemolyse	-	-	-	-	-	-	-
Loss of Brush Broders	-	+	-	+	+	+	+
Petitubular inflammation	-	+	-	+	-	+	-
Tubular cell swelling	-	+	-	+	-	-	-
Tubular congestion	-	+	+	+	+	+	+

Tubular Degeneration	-	+	-	-	-	+	-
Tubular Desquamation	-	+	-	-	-	+	-
Tubular necrosis	-	+	-	+	-	+	-

Discussion

In the present investigation, nephroprotective potential of ethanolic leaf extract of *Baccaurea ramiflora* and *Microcos paniculata* at two dose levels (100 and 200 mg/kg) was evaluated by estimation of kidney function test, i.e. by estimating the amount of urea, serum creatinine, RBC and haemoglobin of gentamycin and paracetamol-treated rats and by histological examination of kidney of gentamycin and paracetamol -treated rats.

The results of the present study gives the experimental evidence for ARF nephroprotective effect against gentamycin and paracetamol induced nephrotoxicity in rats. The qualitative phytochemical screening revealed the presence of alkaloids, glycosides, tannins, saponins, proteins and flavonoids. Administrations of gentamycin (80mg/kg i.p.) and paracetamol (750mg/kg i.p.) for 8 days reported to causes nephrotoxicity. The rats administered with gentamycin and paracetamol have shown significant increased levels of serum urea, serum creatinine whereas RBC and haemoglobin levels were significantly decreased. The creatinine is easily filtered by the renal glomerulus. Tubular reabsorption does not occur. The increased serum creatinine level is produced by kidney damage, which leads to a decreasing GFR and serum creatinine filtration. The increase in the serum creatinine levels in the gentamycin and paracetamol treated group is due to decreased GFR caused by the gentamycin and paracetamol. The serum urea accumulates in renal diseases, because the rate of serum urea production exceeds the rate of clearance.¹⁹

Conclusion

In conclusion, ethanolic leaf extract of *Baccaurea ramiflora* and *Microcos paniculata* contain phytoconstituents- flavonoids, tannins which could enhance renal mitochondrial antioxidant system, thereby protecting against gentamycin and paracetamol toxicity. Although, the active principles were not isolated and their possible mechanism of actions was not investigated in this study, these could constitute areas of future studies.

Conflict of Interest: The authors do not have any conflict of interest.

References

1. Tavafi. Inhibitory effect of olive leaf on gentamicin induced Nephrotoxicity in rats. Asian network for scientific information, 2012; 4: 251-56.
2. Newman DJ, Price CP. Renal Function and Nitrogen Metabolites. Tietz Textbook of Clinical Chemistry, 1999; 1204-70.
3. K. Ilango, G. Maharajan, S.Narasimhan. Int J Health Res, 2009; 2(2): 195-199.
4. Porter GA and Bennett WM. Nephrotoxic acute renal failure due to common drugs American journal of Physiology, 1981; 241(7): F1-F8.
5. Singh NP, Ganguli A, Prakash A. Drug-induced kidney diseases. J Assoc Physicians India, 2003;51:970-976.
6. Mohana LS, Usha KR, Sandhya RK. A review on medicinal plants for nephroprotective activity. Asian J Pharm Clin Res, 2012; 5(4):8-14.
7. B Bawara, M Dixit, N S Chauhan, V K Dixit, D K Saraf. International Journal of Phytomedicine, 2010; 2: 01-09.
8. DJ Jollow, SS Thorgeirsson, WZ Potter, M Hashimoto, JR Mitchell. Pharmacology, 1974; 12: 251-71.
9. LT Wong, LW Whitehouse, G Solemonraj, CJ Paul. Toxicity Lett. 1981; 9: 145-51.
10. Zimmeraman M. Ethical guidelines for investigation of experimental pain in conscious animal. Pain, 1983; 16: 109-110.
11. Narviya MB, Parmar P, Shukla VJ, Ravishankar B. Toxicological study of Balakaturbhandrika churna. J Ayurveda Integr Med, 2011; 2: 79-84.
12. Rashid Ali, Raisuddin Ali, Abhinav Jaimini, Dhruv Kumar Nishad, Gaurav Mittal, Om Prakash Chaurasia, et al. Acute and sub acute toxicity and efficacy studies of Hippophae rhamnoides based herbal antioxidant supplement. Indian J Pharmacol, 2012; 44: 504–508.
13. Ugbabe GE, Ezeunala MN, Edmond IN, Apev J, Salawu OA. Preliminary phytochemical, antimicrobial and acute toxicity studies of the stem bark and the leaves of a cultivated Syzygium cumini Linn. Afr J Biotechnol, 2010; 9: 6943-6947.
14. Glauco Morales, Adrian Paredes, Alberto Olivares and Jaime Bravo. Acute oral toxicity and anti-inflammatory activity of hydroalcoholic extract from *Lampaya medicinalis* Phil in rats. Biological Research, 2014; 47: 6.

15. OECD Guidelines for the Testing of Chemicals/Section 4. Health Effects Test No. 425. Acute Oral Toxicity, Up-and-Down Procedure. Paris, Organisation for Economic Co-operation and Development Publishing. 2008.
16. A Jain and A K Singhai, Nat Prod Res, 2010; 24 (15): 1379-89.
17. A Jain, A Nahata and A K Singhai. Sci Pharm, 2013; 8(14): 1071-87.
18. Sahoo Himanshu Bhusan, Swain Sudhanshu anjan, Nandy Subhangankar,Sagar Rakesh and Bhaiji mrita. Nephroprotective Activity of Ehanolic Extract of Elephantopus Scaber leaves on Albino Rats. IRJP, 2012; 3: (5).
19. Kishor K and Singh M. Effect of becosides, an alcoholic extract of *Bacopa monnieri* amnesia in mice. Indian J of Exper Biol, 2005; 43: 640-5.

Corresponding Author:

Suwendu Saha*

Email: suwendu.belonia@gmail.com