Effect of Ethanolic and Aqueous Extracts of *Bauhinia Variegata* Linn. on Gentamicin-Induced Nephrotoxicity in Rats

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ABSTRACT

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The present study was aimed at evaluating the ethanolic and aqueous extracts of root of *Bauhinia variegata* Linn. for antioxidant and nephroprotective effect in gentamicin-induced nephrotoxicity in rats. The antioxidant activity of both ethanolic and aqueous extracts of root of *Bauhinia variegata* Linn. was carried out by *in-vitro* models such as scavenging of free radicals like 1,2-diphenyl1-2-picrylhydrazyl (DPPH), nitric oxide and superoxide. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of gentamicin 100 mg/kg/day for eight days. Ethanolic and aqueous extracts of root of *Bauhinia variegata* Linn. at dose of 200 and 400 mg/kg b.w. were concurrently given by oral route. Serum creatinine, serum urea, urine creatinine and blood urea nitrogen (BUN) were determined on day 9. Histopathological study of kidney was also done. Both ethanolic and aqueous root extracts of *Bauhinia variegata* Linn. produced significant free radical scavenging activity. Both the extracts produced significant nephroprotective activity in Gentamicin induced nephrotoxicity model as evident by decrease in elevated serum creatinine, serum urea, urine creatinine and BUN levels, which was further confirmed by histopathological study. Gentamicin induced glomerular congestion, blood vessel congestion, and epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the groups receiving the root extract of *Bauhinia variegata* Linn. along with gentamicin.

KEYWORDS: Antioxidant, *Bauhinia variegata* Linn., Nephrotoxicity

INTRODUCTION

The plant *Bauhinia variegata* Linn. *(Caesalpiniaceae)* commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It is widely used in folklore medicine. Its bark, root, leaves, seeds and flowers are used for their medicinal properties. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic[1]. The stem contains - sitosterol, lupeol, kaempferol-3-glucoside and 5,7-dihydroxy and 5,7-dimethoxy flavanone-4-O--L-rhamnopyranosyl--D-glucopyranosides. Flowers contain cyanidine-3-glucoside, malvidin-3-glucoside, malvidin-3-diglucoside, and peonidin 3-diglucoside, kaempferol-3-galactoside and kaempferol-3-rhamnoglucoside. Five flavonoids isolated from the different parts of *Bauhinia variegata* has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside. Phytochemical analysis of the root bark of *Bauhinia variegata* Linn was reported to contain a new flavanone: (2S)–5,7-dimethoxy-3'-4'-methylene dioxyflavanone (1) and a new dihydrobenzoxepin 5,6-dihydro-1,7dihydroxy-3,4-dimethoxy-2-methyldibenz (b,f) oxepin[2,3]. *Bauhinia variegata* Linn. stem is reported to have antitumour[4], antimicrobial[5], anti-inflammatory[6], hepatoprotective[7], antihyperlipidemic[8] and immunomodulatory activities[9].

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Free radicals are highly reactive substances formed in the body as a result of metabolic processes. Many of these molecular species are oxygen (and some times nitrogen) centered free radicals and its non radical products[10]. The term "reactive oxygen species" (ROS) collectively denotes oxygen centered radicals (super oxide and hydroxyl radicals) as well as non-radical species derived from oxygen such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous (HOCl) acid. The increased production of ROS seems to accompany most forms of tissue injury. Free radicals can also react with DNA, proteins or lipids in the cell membrane and cause damage[11].

The involvement of ROS in aging and in many chronic diseases has been considered. The defense provided by antioxidant systems is crucial for the survival of organisms. Detoxification of ROS in the cell is provided by both enzymatic and non-enzymatic systems which constitute the antioxidant defense systems. These antioxidants play a role in delaying, intercepting, or preventing oxidative reactions catalysed by free radicals[12].

Aminoglycosides have long been one of the common causes of drug induced nephrotoxicity. Gentamicin is a very effective antibiotic in treating gram-negative bacterial infection in both humans and animals. Gentamicin induced nephrotoxicity is a

model of acute renal failure caused by oxidative stress generated through the induction of superoxide[13]. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localised mainly in the proximal tubules. It is a complex phenomenon characterized by an increase in plasma creatinine and urea levels and severe proximal tubular necrosis, followed by deterioration and renal failure[14].

The toxicity of gentamicin is believed to relate to generation of reactive oxygen species (ROS) in kidney. Several reports have documented the pathogenesis of aminoglycosides-induced renal tubular cell injury such as derangement of lysosomal, mitochondrial and plasma membrane structure. Furthermore results of many studies have been shown that the altered concentrations of various biochemical indicators of oxidative stress in kidney tissue are due to gentamicin. Because of the obvious mediation of ROS in gentamicin induced renal damage, several antioxidant agents have been used to block gentamicin induced nephrotoxicity[15,16].

Extensive pharmacological evaluation has been done using stem bark of *Bauhinia variegata* Linn., where as very less work has been carried out using *Bauhinia variegata* Linn. root. The present study was carried out to evaluate antioxidant and nephroprotective activities of ethanolic and aqueous extracts of *Bauhinia variegata* Linn. root.

MATERIAL AND METHODS

Plant material

The root of *Bauhinia variegata* Linn. was procured and authenticated by Shri A. V. Bhatt, survey officer, Regional Research Institute (Ay.), Bangalore, Karnataka. (voucher specimen no. RRCBI MCW 79/4).

Preparation of the root extract

The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. The powdered drug was defatted by extracting with pet-ether (60-80^0 C). Coarse powder of the root (1 Kg) was soxhlet extracted with 90% ethanol. The aqueous extract was prepared using the same marc by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (4.2%) and the aqueous extracts (2.4 %).

Chemicals

The chemical DPPH (1, 2-diphenyl-2-picryhydrazyl), N-(1-Naphthyl) ethylendiamine dihydrochloride, NADH, phenazine methosulphate, trichloro acetic acid and potassium ferricyanide were purchased from Sigma chemical, St Louis, MO, USA. All other chemicals and reagents used were of analytical grade.

Animals

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±2^{o}c), humidity (55±5%) and 12h light and 12h dark cycles. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee (IAEC) of K. L. E. Society's College of Pharmacy, Bangalore was taken for conducting wound healing activity. (Ref. No. IAEC/KLECP/BNG/06/2009)

Acute toxicity studies

Acute toxicity studies for aqueous and ethanolic extracts of *Bauhinia variegata* Linn. were conducted as per OECD guidelines 423 using albino Wistar rats. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and up to 24h for mortality.

In-vitro antioxidant models

For the *in-vitro* antioxidant models mentioned below, ascorbic acid and butylated hydroxy anisole were used as a reference standards.

DPPH radical scavenging activity

DPPH radical scavenging activity of the extracts was measured by the method of Shimada *et al*[17]. Various concentrations of the extracts (10-50 g/ml) in distilled water were added to solution of DPPH (1ml, 0.1mM in ethanol) and the mixture was shaken vigorously. After placing it in room temperature for 30 min, absorbance was measured at 517 nm.

Superoxide radical scavenging activity

Superoxide radical scavenging activity was determined by the method of Yen and Chen[18]. To various concentrations of extracts (100-500 g/ml), phenazine methosulphate (1ml, 60 M in phosphate buffer pH 7.4), NADH (1ml, 450 M in phosphate buffer, pH 7.4) and NBT (1ml, 300 M) were added and incubated at 25^0 C for 5 min. The absorbance was recorded at 560 nm against blank.

Butylated hydroxy anisole (BHA) and ascorbic acid were used as standards for the various *in-vitro* antioxidant studies.

Nitric oxide radical scavenging activity

It was determined by adding different concentrations of the extracts (100-500 g/ml) to 0.6 ml sodium nitroprusside and phosphate buffered saline. The solution was incubated at 25^0 C for 120 min. To 1 ml of the incubated solution, 1ml of Greiss reagent (1% sulphanilamide and 0.1% naphthyl ethylene diamine di hydrochloride in 2% phosphoric acid) was added and absorbance was measured at 546 nm against blank solution[17].

The percentage scavenging of various radicals were calculated using the formula

$$\% \text{ radical scavenged} = (A_0 - A_1)/ A_0 X100$$

Where, A_0 is absorbance of the free radical alone and A_1 is absorbance of free radical in presence of extract/standard. All the experiments were performed in triplicate. The IC50 values (Mean±S.E.M in µg/ml) for the various *in-vitro* antioxidant models was determined by regression analysis.

Gentamicin induced nephrotoxicity in rats

Albino rats (150-200 g) of either sex were used for the study. Animals were divided into six groups, each containing six animals. The study was carried out for nine days and treatment was given for eight days. Group I served as control group and received distilled water p.o. for eight days. Group II served as gentamicin group. The gentamicin treated group received 100 mg/kg/day gentamicin by the intraperitoneal (i.p.) route. Group III and IV received 200 and 400 mg/kg b.w. of aqueous extract of *Bauhinia variegata* Linn. root (BVRA) respectively. Group V and VI received 200 and 400 mg/kg b.w. of ethanolic extract of *Bauhinia variegata* Linn. root (BVRE) respectively.

Animals of groups III to VI were administered 100 mg/kg b.w. of gentamicin i.p. along with extracts p.o. for 8 days. After dosing on the day 8, individual rats were placed in separate metabolic cages for 24h for urine collection to determine urine creatinine content. Blood samples were collected via retro-orbital puncture at the end of 24h, the serum was rapidly separated and processed for determination of serum creatinine, serum urea, blood urea nitrogen (BUN), using of Span Diagnostic kits. Body weight of animal was also recorded. Rats were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination[19].

RESULT AND DISCUSSION

There was no change in normal behavioral pattern of animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first 2h and then observed up to 24h for mortality. The extracts were safe up to a maximum dose of 2000 mg/ kg b.w. The pharmacological evaluation was carried out at doses of 200 and 400 mg/kg b.w by oral route.

An *in-vitro antioxidant* study is based on the ability of the extracts to scavenge free radicals. The results of *in-vitro* antioxidant studies indicate that both ethanolic and aqueous extracts of root of *Bauhinia variegata Linn.* possesses significant antioxidant potential. The IC50 values (Mean±S.E.M in µg/ml) for the various *in-vitro* antioxidant models was are indicated in Table No 1.

DPPH radical scavenging activity

The various extracts produced significant DPPH radical scavenging activity from 10 g/ml. The IC_{50} (mean ± S.E.M.) of the various extracts were compared to that of the standards-Ascorbic acid (AA) and Butylated hydroxyl anisole (BHA) as shown in Table No 1. The DPPH radical is lipophilic, relatively stable nitrogen centered free radical that easily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents, as a result of which the electrons become paired off forming the corresponding hydrazine. The solution therefore loses colour stoichiometrically depending on the number of electrons taken up[20]. Hence, DPPH is usually used as a substrate to evaluate antioxidative activity of antioxidants. The decrease in absorbance of DPPH radical caused by antioxidants is due to the reaction between antioxidant molecules and DPPH radical which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. From the results it is evident that the extracts are acting as hydrogen donors and thus are able to scavenge DPPH free radical[21].

Super oxide radical scavenging activity

Superoxide anion radical generation was inhibited by BHA (standard) and extracts from 100 g/ml. The IC 50 values of BVRA, BVRE and BHA for scavenging superoxide radical

Tables 1 IC50 values of AA, BHA, BVRA, BVRE for scavenging the free radicals: DPPH, Super oxide and Nitric oxide.

Test/standard groups	Ic_{50} values Mean± S. E. M. (µg/ml) for free radical scavenging activity		
	DPPH	Super oxide	Nitric oxide
Ascorbic acid	30.55 ±0.57	-	-
BHA	29.11± 0.39	435.40± 2.15	368.00 ± 0.90
BVRA	37.73 ±0.72	502.10 ± 2.78	478.80 ± 1.18
BVRE	36.01 ± 0.61	445.30 ± 1.23	415.20 ± 0.98

n=3, Where, BVRA, BVRE and BHA indicate of Bauhinia variegata Linn. root of aqueous extract and ethanolic extract and Butylated hydroxyl anisole respectively.

being 502.10 2.78, 445.30 1.23 and 435.40 2.15g/ml respectively (Table 1).

In-vitro superoxide radical scavenging activity was measured by NBT (Nitro blue tetrazolium) reduction. It is one of the most popular methods for evaluation of antioxidant activity[20]. Superoxide anion radicals are one of the most abundantly produced free radicals and are produced endogenously by flavoenzymes (eg. Xanthine Oxidase) which convert hypoxanthine to xanthine and subsequently to uric acid in ischemia reperfusion. The biologically generated superoxide anion dismutases into molecular oxygen and hydrogen peroxide in the presence of protons. Superoxide and hydrogen peroxide serve as precursors of singlet oxygen and hydroxyl radicals and indirectly instigate lipid oxidation[22].

In the PMS-NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS-NADH coupling reaction, reduces NBT to a blue coloured formazon that can be measured at 560nm[21,22]. Decrease in absorbance at 560nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. The antioxidant properties of flavonoids are found to be mainly via the scavenging of superoxide anions [21]. *Bauhinia variegata* Linn contains flavonoids and in accordance with the above statement exhibited anti-oxidant activity.

Nitric oxide radical scavenging activity

The IC_{50} values of BVRA and BVRE was found to be 478.80 1.18 and 415.20 0.98 g/ml respectively as against 368.00 ± 0.90 g/ml of BHA [Table 1].

Nitric oxide is a short lived free radical generated by endothelium, macrophages and neurons. It exerts influence on a number of functions including vasodilation, neurotransmission, synaptic plasticity and memory in the central nervous system and is an important chemical mediator. Overproduction of Nitric oxide can mediate toxic effects like DNA fragmentation, cell damage and neuronal cell death[23]. Oxygen reacts with the excess NO to generate nitrite and peroxy nitrite anions which act as free radicals. Effect of various extracts on *in-vitro* NO radical scavenging activity was taken up using sodium nitroprusside and phosphate buffered saline solution (PBS) to generate nitric oxide. The generated nitric oxide was measured by using Greiss reagent[24].

Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce a nitrite ion, which can be estimated using Greiss reagent. The compounds that possess NO scavenging activity inhibits nitrite formation by competing with oxygen to react with nitric oxide [23]. This leads to decrease in nitrite concentration in the assay media. The extracts were found to scavenge the Nitric oxide radical at the various concentrations tested.

Nephroprotective Activity

Urine creatinine, serum creatinine, serum urea and blood urea nitrogen were found to be significantly *(P<0.001)* increased in rats treated with only gentamicin, whereas treatment with the aqueous and ethanolic extracts of root of *Bauhinia variegata* Linn. reversed the effect of gentamicin indicating nephroprotective activity. (Table No. 2)

There is a simultaneous significant decrease in the gentamicin-induced nephrotoxicity when the antioxidant defense system is effective. The increased production of ROS in gentamicin-induced nephrotoxicity may be a result of inactivation of antioxidant enzymes such as SOD and GSH-Px. A relationship between nephrotoxicity and oxidative stress has been confirmed by many investigations. The impairment in kidney functions is accompanied by increase in serum creatinine and urea level and kidney tissue MDA levels that indicates lipid peroxidation. It is one of the essential compounds for maintaining cell integrity participation in the cell metabolism[14,25]. The significant and progressive weight loss in gentamicin treated rats may possibly be due to the injury renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight.

Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reaction at small concentration and thereby eliminate the threat of pathological processes. Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidants activity. Root bark of *Bauhinia variegata* Linn is reported to contain polyphenolics (flavonoids and tannins), steroids, saponins and triterpenes. *Bauhinia variegata* has been reported to contain quercetin, rutin, apigenin and apigenin 7-O-glucoside. Flavonoids and quercetin in particular are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules[26]. They show antiatherogenic and anticarcinogenic activities by blocking LDL oxidation and inhibition of processes of bioactivation of carcinogens. Quercetin from *Bauhinia variegata* have been shown to decrease the lipid peroxide formation and restoration of glutathione status and the activities of antioxidant enzymes during gentamicin-induced nephrotoxicity[8]. *Bauhinia variegata* might have exhibited nephroprotective activity by the virtue of its antioxidant activity.

Histopathological Examination

Control animals showed normal glomerular and tubular

Table 2 Nephroprotective activity of ethanolic and aqueous extracts of Bauhinia variegata Linn. in gentamicin induced nephrotoxicity in rats.

Group	Treatment	Serum creatinine (mg/dl)	Serum urea (mg/dl)	Urine creatinine (mg/dl)	BUN (mg/dl)	Body weight (% Change)
I	Control	0.62±0.16	43.17±0.15	96.71±1.54	19.81±0.56	3.23±0.27
II	Gentamicin	3.11±0.67^{***a}	174.4±1.45^{***a}	281±2.06^{***a}	81.44±1.92^{***a}	-11.68±0.3^{***a}
III	BVRA 200	1.42±0.52^{***b}	102.4±1.23^{**b}	199.2±0.86^{***b}	47.78±1.58^{**b}	-6.83±0.60^{*b}
IV	BVRA 400	1.09±0.43^{***b}	83.6±0.45^{***b}	108.2±0.97^{***b}	36.16±0.63^{***b}	-6.38±0.67^{*b}
V	BVRE 200	0.82±0.10^{***b}	81.61±1.34^{***b}	139.9±0.86^{***b}	38.11±1.40^{***b}	-6.57±1.07^{*b}
VI	BVRE 400	0.66±0.11^{***b}	57.43±0.98^{***b}	101.6±1.22^{***b}	26.8±0.79^{***b}	-3.76±0.41^{***b}

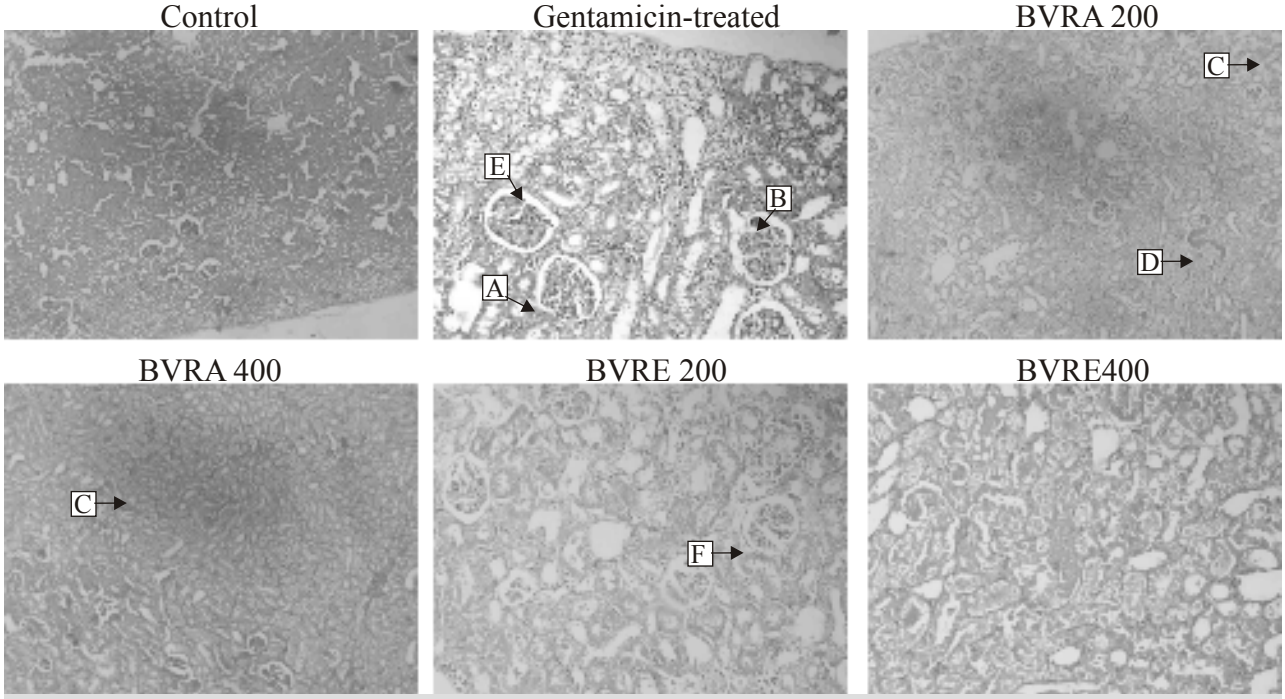
n=6, values are expressed as Mean± S. E. M. BVRA and BVRE 200 and 400 indicates Bauhinia variegata Linn. root aqueous and ethanolic extracts at 200 and 400 mg/kg b.w. respectively. *p<0.05, **P<0.01, ***P<0.001, a-indicates comparison with control, b-indicates comparison with gentamicin treated group.

Table 3: Histopathological features of the kidneys of rats of different treatment groups in gentamicin induced nephrotoxicity.

Histopathological Features -treated	Control	Gentamicin	BVRA 200	BVRA 400	BVRE 200	BVRE 400
Glomerular congestion	-	+++	++	+	+	-
Peritubular congestion	-	+++	++	++	-	-
Blood vessel congestion	-	+++	++	++	++	+
Interstitial edema	-	++	+	+	+	-
Inflammatory cells	-	++	+	-	-	-
Mononuclear infiltration	-	+++	++	+	-	-
Tubular cast	-	+	+	-	-	-

Haematoxylin and eosin stained skin section (200X) were scored as mild (+), moderate (++) and severe (+++) for epidermal and /or re-modeling. BVRA, BVRE 200 and 400 indicates Bauhinia variegata Linn. root aqueous and ethanolic extracts at 200 and 400 mg/kg b.w. respectively.

Fig. 1: Histopathplogical view of renal section from different groups stained with hematoxylin and eosin.



Gentamicin induced nephrotoxicity (Kidney section 200X) A – mononuclear infiltrate cells, B-Tubular casts, C- Interstitial edema, D-Blood vessel congestion, E- Glomerular congestion and F-Peritubular congestion

histology whereas gentamicin produced peritubular and blood vessel congestion. Inflammatory cells were also seen in kidney section from the gentamicin-treated group. In gentamicin group, mononuclear cells infiltrated mainly in the sub-capsular region and interstitial edema was also noticed. Hyaline changes, vacuolization and necrosis in the proximal tubular epithelial cells was also seen. Concurrent treatment with the extracts was found to reduce such changes in kidney histology induced by gentamicin (Fig: 1 and Table No. 3)

Treatment with extracts could prevent cell damage such as tubular vacuolization, glomerular congestion and interstitial edema. According to the pathological result it can be inferred that extracts of *Bauhinia variegata* Linn. had protective effect against degenerative injury caused by gentamicin.

CONCLUSION

The present study revealed that ethanolic and aqueous extracts of *Bauhinia Variegata* Linn. is a good source of phytochemicals with antioxidant properties. The extracts also reversed the nephrotoxicity induced by gentamicin. This indicates that *Bauhinia variegata* Linn. can be used as adjuvant with Gentamicin. By this combination therapy, we can get the therapeutic benefit of the gentamicin without botheration of its prominent side effect, nephrotoxicity. The phytoconstituents flavonoids, tannins, steroids and saponins present in the extracts may be responsible for antioxidant activity. By the virtue of antioxidant activity, *Bauhinia variegata* Linn. might have exhibited nephroprotective activity.

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REFERENCES

1. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products. Vol 2 CSIR New Delhi: 1959. p. 56-7.
2. Yadava RN, Reddy VM. A new flavone glycoside 5-hydroxyl 7,3 ,4 ,5 -tetramethoxy flavones 5-O-β-D-xylopyronosyl (1 2) α-L-rhamopyroanoside form *Bauhinia variegata* Linn. J Asian Nat Prod Res 2001;3:341-6
3. Reddy MV, Reddy MK, Gunasekar D, Caux C, Bado B. A flavonone and a dihydrodibenzoxepin from *Bauhinia variegata*. Phytochemistry2003;64:879-82.
4. Rajkapoor B, Jayakar B, Murugesh N. Antitumor activity of *Bauhinia variegat*a Linn. on Dalton's ascytic lymphoma. J Ethnopharmacol 2003;89(1):107-9.
5. Sharma RN, Saxena VK. In vitro antimicrobial efficacy of leaves extracts of *Bauhinia variegata* Linn. Asian J Chem 1996;8(4):811-2.
6. Yadava RN, Reddy VM. Anti-inflammatory activity of a novel flavanol glycoside from *Bauhinia variegata* Linn. Nat Prod Res 2003;17:165-9.
7. Bodakhe SH, Ram A. Hepatoprotective properties of *Bauhinia variegata* bark Extract. Yakugaku Zasshi 2007;127(9):1503-7.
8. Rajani GP, Purnima A. In vitro antioxidant and antihyperlipidemic activity of *Bauhinia variegata* Linn. Indian J Pharmacol 2009 oct;41(5)227-32.
9. Ghaisas MM, Shaikh SA, Deshpande AD. Evaluation of the immunomodulatory activity of ethanolic extract of the stem bark of *Bauhinia variegata* Linn. Int J Green Pharm 2009;3:70-4.
10. Halliwell B, Gutteridge JM, Cross CE. Free radicals, antioxidant and human disease: where are we now? J Lab Clin Med 1992;119:598-620.
11. awkins CL, Brown BE, Davies MJ. Hypochlorite and hypobromite-mediated radical formation and its role in cell lysis. Arch Biochem Biophys 2001;395(2):137-45.
12. McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000;108:652-9.
13. Maldonadu PD, Barrera D, Rivero I, Mata R, Copos ON, Pando RH. Antioxidant S-allo cysteine prevents gentamicin-induced oxidative stress and renal damage. Bio med 2003;35:317-24.
14. Silan C, Uzuno O, Comunoglu NU. Gentamicin-induced nephrotoxicity in rats ameliorated and healing effects of resveratrol. Biol Pharm Bull 2007;30(1):79-89.
15. Yaman I, Balikci E. Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol 2010;62(2):183-90.
16. Reiter RJ, Tan D, Sainz RM, Mayo JC, Lopez-Burillo S. Melatonin: reducing the toxicity and increasing the efficacy of drugs. J Pharm Pharmacol 2002;54:1299-1321.
17. Gulcin I, Alici HA, Cesur M. Determination of *in-vitro* antioxidant and radical scavenging activities of propofol. Chem Pharm Bull 2005;53(3):281-5.
18. Kaur G, Alam MS, Jabbar Z, Athar M. Evaluation of antioxidant activity of *Cassia siamea* flowers. J Ethnopharmacol 2006;108:340-8.
19. Harlaka GV, Patil CR, Patil MR. Protective effect of *Kalanchoe pinnata* pers. *(Crassulaceae)* on gentamicin-

induced nephrotoxicity in rats. Indian J Pharmacol 2007 August;39(4):201-5.

20. Blois MS. Antioxidant activity of grape seed extracts on peroxidation models *in –vitro*. J Agri Food Chem 2001;55:1018.
21. Kumar RS, Shivakumar T, Sunderam RS, Gupta M, Mazumdar UK, Gomathi P, *et al*. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Braz J Med Bio Res 2005;38:1015-24.
22. Shrikumar S, Sukumar M, Ravi TK. *In- vitro* antioxidant activity of various extracts of *Curculigo orchioides gaertn* (Fam: *Amaryllidaceae*). J Nat Rem 2007;7/2;252-7.
23. Sumanont Y, Murakami Y, Tohda M, Vajragupta O, Matsumoto K, Watanabe H. Evaluation of nitric oxide radical scavenging activity of manganese complexes of curcumin and its derivative. Bio Pharm Bull 2004;27(2):170-3.
24. Mangathayaru K, Sravan K, Reddy PK, Kumar RM, Swetha B, Reddy CU. *In- vitro* antioxidants studies of the aerial parts of *Origanum majoram* linn. and *Artemesia sieversiana ehrh*. Phcog Mag 2007;3(10):90-3.
25. Kumar KV, Naidu MU, Shifow AA, Ratnakar KS. Probucol protects against gentamicin-induced nephrotoxicity in rats. Indian J Pharmacol 2000;32:108-13.
26. Maldonadu PD, Barrera D, Rivero I, Mata R, Copos ON, Pando RH. Antioxidant S-alllcystein prevents gentamicin-induced oxidative stress and renal damage. Bio med 2003;35:317-24.

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