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Effect of *Ficus benghalensis* L. Latex Extract (FBLE) on Cisplatin Induced Hypotension and Renal Impairment in Wistar Rats

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Abstract

Background and objective: *Ficus benghalensis* is a remarkable tree that sends down its branches and great number of shoots. It is used for treatment of neuralgia, rheumatism, lumbago, bruises, nasitis, gonorrhoea, inflammations, cracks of the sole and skin diseases and in ayurveda for diarrhea, dysentery, and piles". To evaluate protective activity of *F. benghalensis* latex extract (FBLE) on cisplatin induced hypotension and renal impairment in wistar rats.

Materials and methods: Rats were divided five groups and duration study 16 days. 1st group administered 5 ml/kg normal saline; 2rd group FBLE treated group 400 mg/kg per day; 3rd group (cisplatin treated) with single dose of cisplatin (5 mg/kg, i.p.) on 1st day and keep animals up to 6 days; 4th Group and 5th Group FBLE treated (200 and 400 mg/kg, p.o.) of for 1st to 10th day and single dose of cisplatin (5 mg/kg, i.p.) on 11th day.

Results: Phytochemical screening of FBLE has revealed presence of glycoside, alkaloids, tannin, flavonoids and amino acids, IC_{50} values for DPPH, and phosphor-molybdenum were 28.63 µg/ml ± 0.16 µg/ml, and 31.84 µg/ml ± 0.12 µg/ml respectively. The cisplatin-treated 3rd group showed a significant (**P<0.01) changes renal functions biochemical parameters, blood pressure and histopathology were significantly (**P<0.01) monitored by 200 mg/kg and 400 mg/kg protective groups.

Conclusion: These findings demonstrated that the FBLE and their constituents have excellent nephroprotective and normalized blood pressure.

Keywords: *Ficus benghalensis;* latex; Cisplatin; Hypotension; Renal impairment

Introduction

Ficus benghalensis is a remarkable tree from India that sends down its branches and great number of shoots, which take root and become new trunks [1]. Its chemical constituent's flavonoids leucoanthrocyanidin, leucoanthocyanin, friedelin, β sitosterol, querecitin-3-glactoside and rutin. Earlier, glucoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one, beta sitosterol-alpha-D-glucose, and meso-inositol have been isolated from the bark of the *F. benghalensis* and it latex contains Caoytchoue (2.4%), Resin, Albumin, Cerin, sugar, and Malic acid [2] and used for treatment of neuralgia, rheumatism, lumbago, bruises, nasitis, gonorrhoea, inflammations, cracks of the sole and skin diseases [3] and in ayurveda for diarrhea, dysentery, and piles [4]. The extract of *F. benghalensis* was reported to inhibit insulinase activity from the liver and kidney and it was also found to inhibit the lipid peroxidation. *F. benghalensis* was traditionally used for the treatment of mehavikar or urinary disorders [5] but no scientific studies have been undertaken to verify these claims. Thus, the purpose of current study was to investigate whether oral administration of *F. benghalensis* latex extract has possible protective effect against cisplatin induced hypotension and renal impairment in wistar rats.

Materials and Methods

Phytochemical standardization

Phytochemical identification and standardization of FBLE performed by TLC Method and HPTLC (CAMAG Switzerland, Linomet 5, and Scanner 3, Win Cat Software) Mobile phase: Butanol: Formic acid: Water (7.5 ml: 1.5 ml: 1.0 ml). HPTLC analysis performed by use of various standard amino acid markers like glutamine, glycine, cysteine, methione, lysine, arginine etc., and extract in which one compound was identified on the extract track and their RF value 0.56 was similar to standard methionine marker. The methionine content of FBLE standardized that was found 0.842 ± 0.0364 % of standard methionine.

Determination of total phenolic and flavonoid contents in FBLE

The total Phenolic and Flavonoid content of latex extract determined by method [6,7] respectively.

In vitro antioxidant activity

In vitro antioxidant studies of FBLE evaluated by Method DPPH [8] ferric chloride [9] phosphor-molybdenum [10] free radical scavenging.

Animals

Adult male Wistar rats (180-210 g) have an access to water and food ad libitum, and maintained under constant (25 ± 1°CAS), humidity (65 ± 10%) and a 12 h light/dark cycle. The experiment was carried out in accordance to the guidelines mentioned in the CPCSEA, and IAEC approved the experiment protocols (SVU/PH/IAEC/26.03.2010/02).

Acute toxicity study

Each group of Wistar rats fasted overnight prior to the experiment. Each group of rats fed FBLE dissolved in normal saline with increasing

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dose like 5, 50, 100, 200, 400, 1000 2000 mg/kg body weight. The animals observed continuously for 2 h and then every 2 h up to 24 and 72 h for gross behavior changes. So LD_{50} cut off of the extract was 2000 mg/kg body weight. FBLE dose regimen prepared like 1/10th and 1/5th of the respective LD_{50} cut off values.

Cisplatin-induced renal injury

Five groups of rats (n=6) used, in which 1st group administered 5 ml/kg normal saline throughout the experiment for 16 days; 2rd control group received FBLE 400 mg/kg per day for 16 days; 3nd group (cisplatin treated) with single dose of cisplatin (5 mg/kg, i.p.) on 1st day and keep animals up to 6 days; 4th Group and 5th Group (Protective) FBLE (200 and 400 mg/kg, p.o. for 1st to 10th day and single dose of cisplatin (5 mg/kg, i.p.) on 11th day and keep animals up to 16 days [11].

On the 6th day in cisplatin control and 16th day in control, protective were measured blood pressure by help of student physiograph (instruments & chemicals PVT. LTD, Ambala, India) after then blood withdrawn from retro-orbital sinus of rats for biochemical estimation for serum urea and creatinine levels using diagnostic kit from Span Diagnostic, "Kolkata on chemical analyzer (Microlab 3000) and also dissected out the kidneys for estimation of *in vivo* antioxidant enzymes and histopathological works [12]."

In vivo Antioxidant activity

Rat kidneys homogenized and centrifuged at 10,000 rpm at 0°C for 20 min. The supernatant used for estimation of antioxidant enzymes level by calorimetric method using spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam), Glutathione reductase (GSH) estimated by method [13] Lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) methods [14] Superoxide dismutase (SOD) by method [15] Catalase (CAT) by colorimetric assay [16] and the sediment of the centrifuge used for estimation of the Na^+K^+ATPase by method [17] Ca^{2+} ATPase [18] Mg^{2+} ATPase [19].

Statistical analysis

Result were expressed as mean ± SEM, Statistical Analysis were performed with one way analysis of variance (ANOVA) followed by Dunnett's test. P value less than <0.05 was considered significant.

Results

FBLE has revealed presence of glycoside; alkaloids, tannin (Phenolic compound), Flavonoids, and methionine amino acid (Figures 1 and 2). Total Phenolic and flavonoids content had obtained 2.76 ± 0.84 mg GAE/g and 1.84 ± 0.5 mg QE/g extract respectively.

In vitro antioxidant potential of FBLE was evaluated by scavenging effect of DPPH, ferric chloride, and phosphor-molybdenum. IC_{50} values for DPPH, and phosphor-molybdenum were 28.63 ± 0.16 µg/ml, and 31.84 ± 0.12 µg/ml respectively (Figures 3 and 4).

Blood pressure of cisplatin-treated was decreased to 70 mmHg which is significantly increased 100 mmHg in protective groups.

The cisplatin-treated showed a significant increase urine volume, serum urea and creatinine levels, lipid peroxidation and decrease body wt. (Figure 2), GSH, SOD, CAT, $Na^{+/}K^+$ ATPase, Ca^{++} ATPase, Mg^{++}ATPase of kidney (Tables 1-3), on the 6th day as compared to the group I. They were significantly ($p<0.01$) recovered in protective regimen with treated dose at 200 and 400 mg/kg of FBLE.

Histopathological sections of the kidneys showed marked vasoconstriction, hyaline droplets, proinflammatory and tubular necrosis were observed cisplatin treated group III (Figure 5; Plates 1A-1C) and in the protective regimen extract (200 and 400 mg/kg body wt., p.o.) reduced hyaline droplets, tubular dilation and recovery of tubular necrosis in which 400 mg/kg more effective reduction than 200 mg/kg (Figure 5; Plate 1D and 1E) respectively.

Discussion

In the present study, cisplatin-induced renal impairment was evidenced by an increase in serum urea and creatinine and acute tubular necrosis. These changes observed on 6th day after administration of a single dose 5 mg/kg cisplatin. FBLE normalized, raised serum urea, creatinine levels, lipid peroxidation and decreased blood pressure, GSH, SOD, CAT, $Na^{+/}K^+$ ATPase, Ca^{++} ATPase, Mg^{++}ATPase of kidney. The histopathological report supported the biochemical findings.

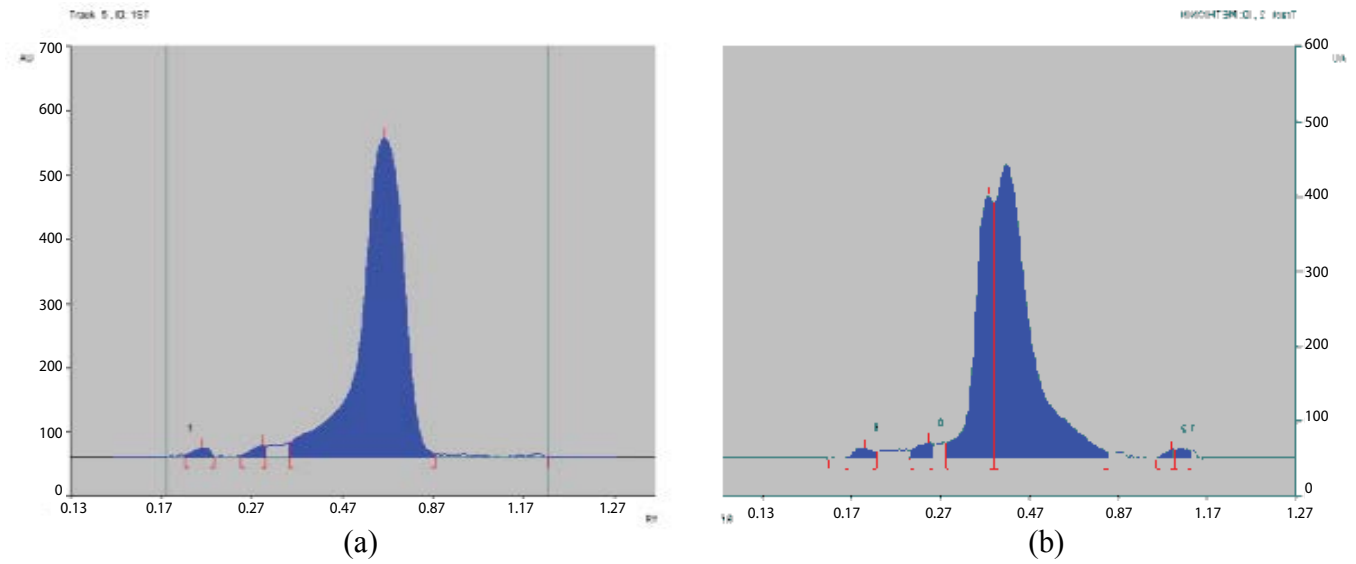


Figure 1: HPTLC chromatogram (a) standard methionine RF= 0.57; (b) HPTLC chromatogram extract RF=0.56.

Groups	Urine volume (ml/24 h)	Urea level in serum (mg/dl)	Creatinine level in blood serum (mg/dl)
Control	5.33 ± 0.33	24.16 ± 1.04	0.94 ± 0.05
Extract FBL	6.66 ± 0.66^{b*}	25.18 ± 1.85^{b*}	0.96 ± 0.03^{b*}
Cisplatin treated	14.66 ± 0.88^{a}	76.66 ± 2.24^{a}	2.32 ± 0.10^{a}
Protective (200 mg/kg)	8.66 ± 0.36^{b*}	50.66 ± 2.82$^{b'}$	1.53 ± 0.01^{b*}
Protective (400 mg/kg)	10.66 ± 0.42^{b**}	61.66 ± 1.05^{b**}	1.85 ± 0.08^{b**}

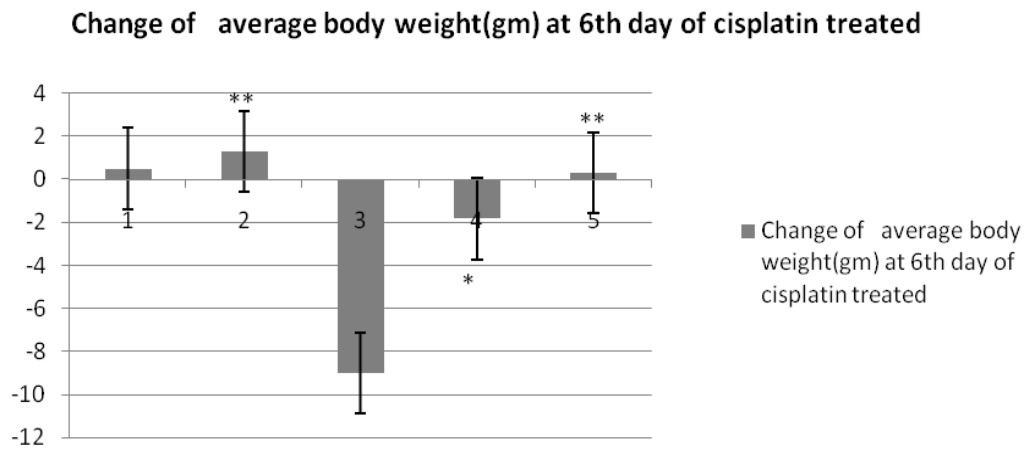
^{a}: P<0.01 as compared to the control; ^{b}: **P<0.01 as compared to the cisplatin treated group; ^{b}: *P<0.05 as compared to cisplatin treated group.

Table 1: Effects of methanol extract of *Ficus benghalensis* latex L. on the Urinary volume, Urea and Creatinine level in serum on 6th day after cisplatin administration.

Groups	µmol GSH/g	n Mol MDA/g. Ml	Unit SOD/g	CAT (µ mole of H_2O_2/g
Control	69.50 ± 1.54	14.00 ± 0.57	21.83 ± 0.94	323.33 ± 1.75
Extract FBL	68.34 ± 2.28^{b*}	14.98 ± 0.36^{b*}	20.43 ± 0.59^{b*}	319.53 ± 5.24^{b*}
Cisplatin treated	45.33 ± 1.66^{a}	24.50 ± 0.61^{a}	07.16 ± 0.60^{a}	201.67 ± 3.33^{a}
Protective (200 mg/kg)	58.66 ± 2.82^{b*}	15.00 ± 2.39^{b*}	15.83 ± 0.60$^{b'}$	285.83 ± 8.00^{b*}
Protective (400 mg/kg)	59.51 ± 2.44^{b**}	18.16 ± 0.74^{b**}	12.66 ± 0.66^{b**}	232.50 ± 4.42^{b**}

^{a}: P<0.01 as compared to the control; ^{b}: **P<0.01 as compared to the cisplatin treated group; ^{b}: *P<0.05 as compared to cisplatin treated group.

Table 2: Effect of methanol extract of *Ficus benghalensis* latex L. on the lipid peroxidation and antioxidant enzymes of kidney on 6th day after cisplatin administration.



1: Control; 2: Extract FBL; 3: Cisplatin treated; 4: Protective 200 mg/kg; 5: Protective 400 mg/kg, dose of extract

Figure 2: Effect methanol *Ficus benghalensis* L. latex extract on average change body weight of various groups as compared to cisplatin treated group (3). Each group represents mean ± S.D. of six animals, **P<0.01,*P>0.05, **P>0.01, **P<0.01, **P>0.01 as compared to the cisplatin treated group.

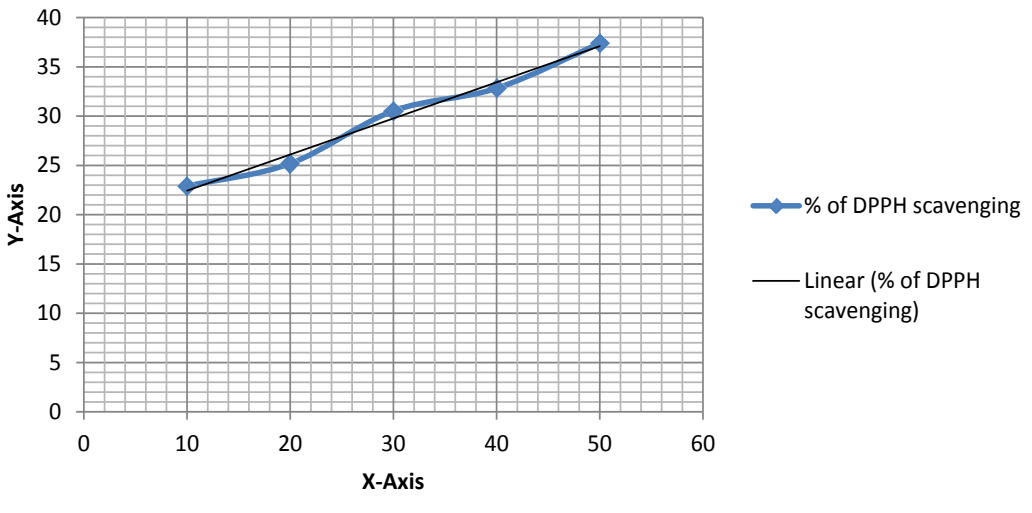


Figure 3: % DPPH radical scavenging activity of FBLE.

Groups	Na^{+}/K^{+} ATPase (mM of phosphate librated/mg tissue)	Ca^{++} ATPase (mM of phosphate librated/mg tissue	Mg^{++} ATPase (mM of phosphate librated/mg tissue)
Control	210.83 ± 2.64	102.83 ± 2.31	152.67 ± 0.88
Extract FBL	210.34 ± 2.37[b*]	101.51 ± 2.31[b*]	154.66 ± 1.66[b*]
Cisplatin treated	135.17 ± 2.51[a]	64.33 ± 1.05[a]	81.66 ± 1.05[a]
Protective (200 mg/kg)	159.83 ± 2.06[b*]	71.53 ± 2.25[b']	98.00 ± 2.19[b*]
Protective (400 mg/kg)	186.56 ± 2.46[b**]	98.28 ± 2.16[b**]	116.16 ± 2.56[b**]

[a]: P<0.01 as compared to the control; [b]: **P<0.01 as compared to the cisplatin treated group; [b']: *P<0.05 as compared to cisplatin treated group.

Table 3: Effects of methanol extract of *Ficus benghalensis* L. latex on ATPase in kidney tissue of various groups on 6th day after cisplatin administration.

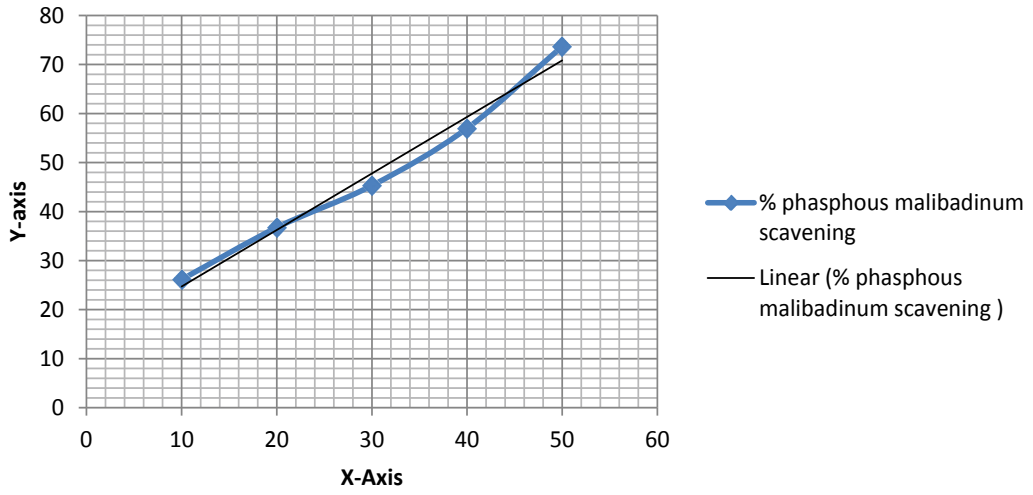


Figure 4: % Phosphor-molybdenum radical scavenging activity of FBLE.

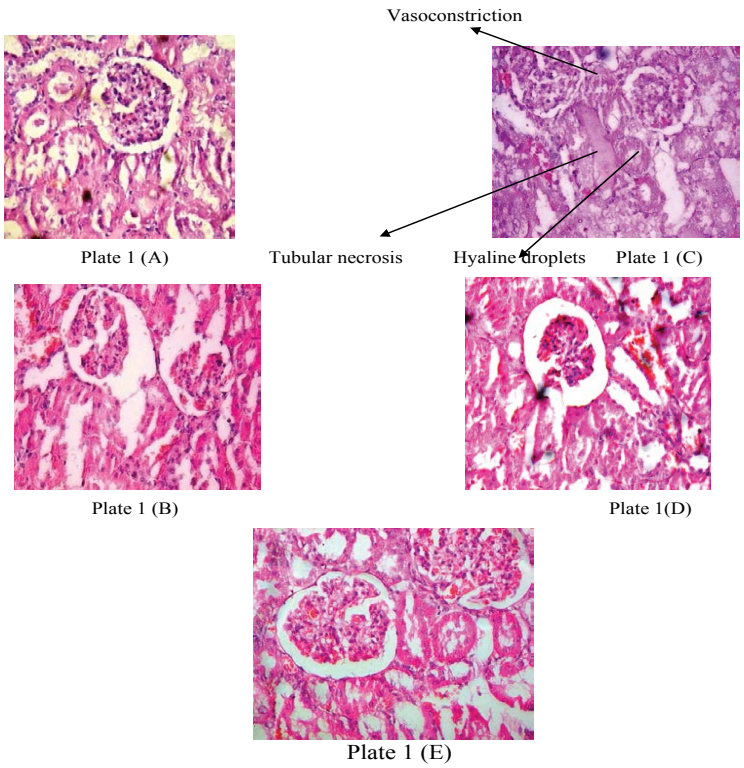


Figure 5: "Histopathology sections of kidney; Plate 1(A) Vehicle-treated, Plate 1 (B) FBLE treated, Plate 1 (C) Cisplatin-treated rats (5 mg/kg, 6 days), Plate 1 (D) Protective (200 mg/kg), and Plate 1 (E) Protective (400 mg/kg)".

The FBLE treated dose 400 mg/kg body weight was observed more significant than 200 mg/kg body wt.

According to previous findings, it was conformed that the single dose of cisplatin (5 mg/kg, i.p.) causes a significantly increase in two serum markers of the kidney function, viz. serum urea and creatinine [20]. Present study was revealed that significantly decrease the level of urea and creatinine in blood serum after treatment with FBLE that was indicate FBLE has nephroprotective activity. A relationship between oxidative stress and nephrotoxicity had well demonstrated in many experimental models Evidence point. The regulation up to normal blood pressure could be protective effect of renal impairment and activation sympathetic nerves system.

In vitro studies of FBLE evaluated for its good antioxidant potential revealed DPPH, ferric chloride, phosphor-molybdenum of free radical scavenging effect with lower IC_{50} values. FBLE has been found to be a rich source of Caoytchoue (2.4%), Cerin, and Malic acid and present phytochemical data have been revealed tannin, Flavonoids, methionine. Yadav [21] also reported hepatoprotective effect of *Ficus religiosa* latex on cisplatin induced liver injury in Wistar rats.

Deegan et al. [22], also reported that nephrotoxicity, cytotoxicity and renal handling of a cisplatin methionine complex in male wistar rats. The present phytochemical screening data has observed methionine which was antagonized cisplatin nephrotoxicity.

Conclusion

Finally, it is concluded that FBLE could be an ameliorated cisplatin hypotension and nephrotoxicity.

Conflicts of Interest

Author declares no conflicts of interest.

Acknowledgement

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