Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [11] October 2014:16-21

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Ethanolic extract of *Ficus religiosa* prevents Cisplatin toxicity by Enhancing antioxidant status in Mice

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ABSTRACT
The clinical use of Cisplatin, has been limited due to the major side effects such as nephrotoxicity and hepatotoxicity. Recent reports have shown that the impairment of antioxidant defense systems is the main offender for side effects of cisplatin. The present study was undertaken to assess the protective effect of aqueous extract of Ficus religiosa, leaf against Cisplatin-induced oxidative dysfunction in mice. Three different doses of Ficus religiosa, leaf (250, 500 and 1000 mg/kg) were administered daily for fifteen days and Cisplatin was administered intra-peritoneally in 3 days interval. The animals were sacrificed 24 h after the last treatment. The liver and kidney were prepared for the biochemical investigations. Cisplatin significantly induced oxidative stress, ultimately leading to increased serum levels of liver enzymes such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and Lactate dehydrogenase. Renal toxicity also characterized by a significant increase in concentrations of serum creatinine and blood urea nitrogen. Supplementation of Ficus religiosa ameliorated the side effects of Cisplatin referenced to improved antioxidant status of liver and kidney. The results of this study concluded that the extract of Ficus religiosa leaf could be proposed to protect the liver and kidney damage induced by Cisplatin. This protective effect might be correlated with the antioxidant properties of leaves extract of Ficus religiosa.
Keywords: *Ficus religiosa, Cisplatin, Oxidative stress.*

Received 01.05.2014 Revised 29.05.2014 Accepted 10.09. 2014

INTRODUCTION

Cisplatin is an effective chemotherapeutic agent used for the treatment of many solid tumors [1]. However, clinical usage of cisplatin is limited by its side-effects such as nephrotoxicity and hepatotoxicity [2]. Oxidative stress has been reported to be involved in the mechanism of Cisplatin toxicity[3].

Ficus religiosa, family Lauraceae, has been extensively used in the traditional medicine for various disorders. It has been shown to exhibit diverse biological activities including wound healing [4], anti-convulsant [5] and anti-inflammatory [6].

The leaf extract of *Ficus religiosa* contains a large number of bioactive components such as polyphenols and flavonoids [7]. The mechanisms of action of polyphenols go beyond the modulation of oxidative stress [8]. Despite the possible anti-oxidative potential of *Ficus religiosa* against side effects of Cisplatin has not been reported so far. Therefore, this study aimed to evaluate the anti-oxidative potential of *Ficus religiosa*, by using the side effects induced by Cisplatin.

MATERIALS AND METHOD

Reagents and laboratory wares

All reagents used were analytical grade. All the diagnostic kits were purchased from Crest Biosystems Company, India. Cisplatin and chemicals were purchased from Sigma Chemical Co., (St. Louis, MO, USA).

Plant material and preparation of extract

Leaf samples of *Ficus religiosa* were collected from botanical garden of University of Pune, Pune, India in SEP-2013. The plants were identified and authenticated by a botanist from the department of botany, Pune University, India.

The leaves were washed and oven dried at 50°C. The dried material was then pulverized into powder by a mechanical grinder and stored at -20°C.

Ground powder (250 gm) were subjected to 500ml of ethanol (90%), kept on a shaker for 24 hrs and filtered through three layer of muslin cloth. Supernatant was then evaporated under reduced pressure in Rotary evaporator at 40°C to dryness. Ethanol extracts stored at -20°C for further use.

Animal and treatments

Thirty female albino mice weighing 30.0±2.0 g, prepared from the animal laboratory of Zoology department of Pune University. Animal room was kept at the temperature of 20±2 ˚C with a 12 h light/dark cycle and humidity of 50–60% with free access to food and water. Mice were housed five per cage in the plastic cages with wood shaving bedding. After one week adaptation period, animals were divided into five groups of five mice each.

All extract dosage was administered daily to animals by forcible feeding (gavage) for fifteen days and Cisplatin (5mg/kg) was injected intra peritoneally (IP) in 3 days interval, as follows:

Group 1: Served as the normal control and received only normal saline (1 mL/kg daily, intraperitoneally)

Group 2: Administrated with Cisplatin only, as the negative control group

Group 3: Administrated with 250 mg/kg *Ficus religiosa* + Cisplatin injection

Group 7: Administrated with 500 mg/kg *Ficus religiosa* + Cisplatin injection

Group 8: Administrated with 1000 mg/kg *Ficus religiosa* + Cisplatin injection

The Institutional Animal Care and Ethics Committees in Pune University, Pune, India approved the protocol of this animal experiment.

At the end of treatment, 24 h after the last dose administration, mice were anesthetized with chloroform.

Sample preparations

Blood samples was collected directly from heart using sterile disposable syringes and immediately transferred into disposable tubes, centrifuged at 1500 rpm for 15 min, serum was collected and stored at -80 ºC for further studies.

The liver and Kidney were removed, washed in ice-cold saline to remove the blood then homogenized (10 ml/gm of tissue) with 0.05 M phosphate buffer (pH 7). The suspension was centrifuged at 1500 rpm at 4ºC for 15 min, and clear supernatant was stored at 80 ºC for further determinations.

Measurement of the serum levels enzyme markers

Alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), total Bilirubin, blood urea nitrogen (BUN) and Creatinine (Cr), contents were estimated by standard commercially kits according to the standard protocol.

The level of lipid peroxidation was measured using tetramethoxypropane and 1'1'3'3'-tetra-methoxypropane as standard [9].

Protein content was determined [10] and bovine serum was used as standard.

Antioxidant Enzyme Studies

CAT activity was measured by a UV colorimetric method [11] using H_2O_2 as substrate; SOD activity was determined according to a colorimetric method [12] from the ability of homogenate to scavenge the superoxide anion generated from the photo-illumination of riboflavin and Reduced GSH were assayed by a standard method [13] based on the formation of a yellow colored complex with [5,5'-Dithio-Bis (2-Nitrobenzoic Acid)].

Statistical analysis

All data were represented as mean ± SD. Data were statistically analyzed by using one-way analysis of variance (ANOVA) followed by the Student's t-test. P values less than 0.05 were considered as significant using SPSS software (Version 16).

RESULTS

Effect of treatments on liver and kidney

Biochemical analysis of serum signified that, cisplatin treatment (Group II) significantly ($P < 0.05$) raised the serum levels of liver enzyme markers ALT, AST, ALP, total bolirubin and LDH as compared to the control group (Table 1). Injection of cisplatin also result the increased levels of Cr, and BUN in negative control group (Group II) as compared to normal group (Figures 1 and 2).

Interestingly, *Ficus religiosa* demonstrated the significant ($P < 0.05$) protective activity against cisplatin by maintaining the serum levels of ALT, AST, ALP, LDH, total bolirubin, Cr and BUN.

Effect of treatments on lipid peroxidation and enzymatic antioxidant activities

The levels of MDA and antioxidants in both liver and kidneys were shown in Table 1-3, respectively. Cisplatin treatment significantly ($P<0.05$) increased the MDA level, and also decreased ($P<0.05$) the antioxidant levels of liver and kidney. The leaf extract of *Ficus religiosa*, decreased the elevated MDA level and also increased ($P<0.05$) the reduced level of antioxidants in both of the organs.

DISCUSSION

Estimations of liver enzymes in the serum reflect the condition of the liver function. Cisplatin induced hepatotoxicity results in the elevated levels of liver enzymes such as ALT, AST, ALP, LDH and total bilirubin. Elevated levels of these enzyme markers in the serum represent the cellular leakage of these enzymes from the cell membrane of liver [14]. In the present study, ethanolic extract of *Ficus relilgiosa* maintained the serum level of liver enzymes.

Antioxidants have been reported to prevent excessive rises of liver enzymes in serum [15]. The power of *Ficus relilgiosa* to maintenance the level of hepato enzymes could be due to the free radical scavenging property of this extract.

Mechanism of Cisplatin toxicity is related to the depletion of the antioxidant defense systems. Antioxidant enzymes are the first line of defense system against oxidative stress [16].

The most important antioxidant in living cells are SOD, CAT, and GSH [17]. In the present study Cisplatin reduced the antioxidant status of liver and kidney. However, the antioxidant levels in *Ficus relilgiosa* treated groups were higher compared to the negative control group. It is supporting the protective potential of *Ficus relilgiosa* against cisplatin.

SOD is an antioxidant to converting superoxide radicals to H_2O_2 [13]. Kilic et al, demonstrated that Cisplatin induces the oxidative stress by decreasing the intracellular concentration of GSH and CAT [2]. These antioxidants are scavenging the cytotoxic H_2O_2, and react with the other reactive oxygen species.

The increased level of lipid peroxidation, in negative control group, suggested the organ damages due to the failure of antioxidant enzymes. However *Ficus relilgiosa* reduced the level of MDA in liver and kidneys near the normal control group.

In previous studies, elevation of MDA has been reported due to Cisplatin toxicity in liver [18] and kidney [12]. The elevation of lipid per oxidation is an important parameter of oxidative stress and depletion antioxidants presented in cells [19].

showed the significant decrease in plasma albumin due to treatment with Cisplatin [20]. The depletion of serum TP and Alb due to Cisplaitn injection in this study are suggested the initial damage of the endoplasmic reticulum. Administration of FR remarkably prevented toxicity of Cisplatin by reduction of TP and Alb. It can suggest its protective effects against oxidative impairment of endoplasmic reticulum.

The rise in the level of TB in serum is a marker of hepato injury [21]. The ability of FR to reduce the level of TB in the serum suggests its preventing hepato cells by stabilization of biliary function.

Nephrotoxicity is an undesired side effect of chemotherapy. According to report of Pabla [22], a minimum dose of Cisplatin (5 mg/kg body weight) is sufficient to induce nephrotoxicity in mice. Nephrotoxicity in this study was gauged by BUN and Cr, as the biochemical markers employed in the diagnosis of renal damage. Significance increases in BUN and Cr levels in negative control group was the indicator of functional damage to the kidney, which was alleviated by pre and post-treatment with FR. This study suggested that FR could improve the antioxidant status of cells and prevent the cell membrane against lipid peroxidation.

Table1 : Effect of oral administration of F. religiousa extract and cisplatin injection on liver marker enzymes in mice.

Experimental Groups	ALT (U/L)	AST(U/L)	ALP(U/L)	LDH (U/L)
Group 1	26.24± 7.48	83.21± 14.74	24.59± 6.29	33.33± 3.24
Group 2	82.35±11.72*	151.39±10.36*	49.48±12.43*	72.29±6.68*
Group 3	85.16± 10.22	74.41± 18.25	22.61± 9.17	28.13± 6.33
Group 4	54.27± 6.51	68.13± 15.31	26.39± 11.12	31.35± 8.28
Group 5	37.52± 9.83	51.11± 9.43	23.71± 10.20	31.64± 9.41
Group6	69.32±18.92	164.42±13.62	32.09±8.67**	69.42±5.62
Group 7	44.07±15.64**	94.38±20.48**	27.24±4.92**	57.82±7.34**
Group 8	27.18±9.02**	85.66±11.71**	26.31±7.28**	41.54±10.52**

Values are mean ± S.D, n = 6; *Indicate significance compared to control group ($p< 0.05$). **Indicate significance compared to Cis- group ($p< 0.05$). ALT: Alanine amino transferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase

LDH: Lactate Dehydrogenase

Table 2: Effect of aqueous extract of F. *religiousa* on antioxidant parameters in Cis-induced hepato and nephrotoxicity.

Treatment	Group 1	Group 2	Group6	Group7	Group 8
SOD (U/mg protein)					
Liver	21.52±7.34	13.92 ± 3.58*	14.21±2.66	19.64±2.18**	21.24±2.27**
Kidney	19.5 ± 4.25	11.91 ± 2.36*	12.33±5.37	20.44±7.36**	20.34±1.85**
CAT (U/mg protein)					
Liver	59.72 ± 6.91	31.23±6.34*	32.2 7± 0.91	48.36±3.47**	53.23±3.34**
Kidney	47.97±11.34	22.75 ± 3.85*	22.82±1.06	33.17±2.08**	47.74±3.96**
GSH (U/mg protein)					
Liver	3.65±6.18	1.32±1.45*	1.51±2.28	2.37±3.61**	2.72±4.57**
Kidney	3.36±2.14	1.29±1.38*	1.37±2.19	2.28±1.37**	3.64±2.31**

Values are mean ± S.D, n = 6; *Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05). Values are mean ± S.D, n = 6; *Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05). SOD: Super oxide dismutase; CAT: Catalase; GSH: Glutathione

Table 3: Effect of aqueous extract of F. *religiousa* on serum TP, albumin and TB in Cis-induced toxicity mice.

Experimental Groups	TP (g/dL)	Alb (mg/dL)	TB (mg/dL)
Group 1	**6.14± 2.54**	**3.65 ±2.38**	**0.25 ±0.82**
Group 2	**4.62±2.25***	**2.08±2.07***	**1.39±1.28 ***
Group 6	**5.12±1.42****	**2.11±1.08**	**1.25±1.32**
Group 7	**5.68±3.68****	**2.92±1.06****	**0.94±0.94****
Group 8	**5.72±2.65****	**3.30±2.04****	**0.72±1.08****

Values are mean ± S.D, n = 6
*Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05).
Values are mean ± S.D, n = 6 *Indicate significance compared to control group (p< 0.05).
**Indicate significance compared to Cis- group (p< 0.05). TP: Total protein; Alb: Total albumin; TB: Total bilirubin

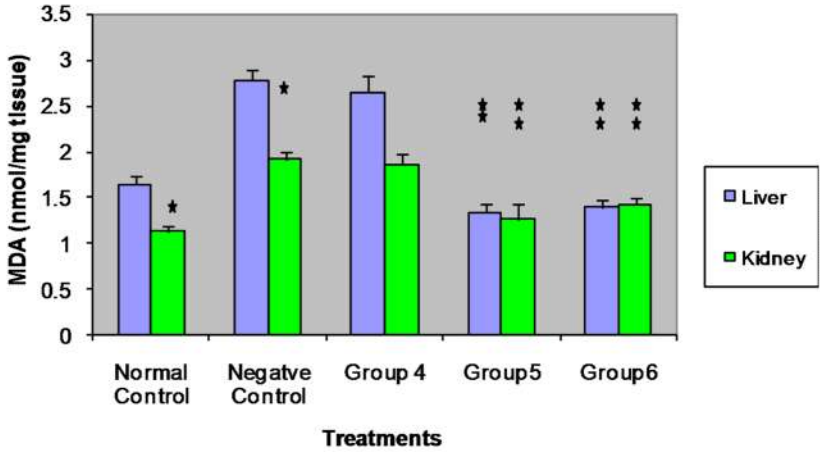

Fig. 1: Effect of aqueous extract F.*religiousa* (FR) on serum MDA level in mice treated with Cisplatin . Values are mean ± S.D, n = 6 *Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05). MDA: Malondialdehyde

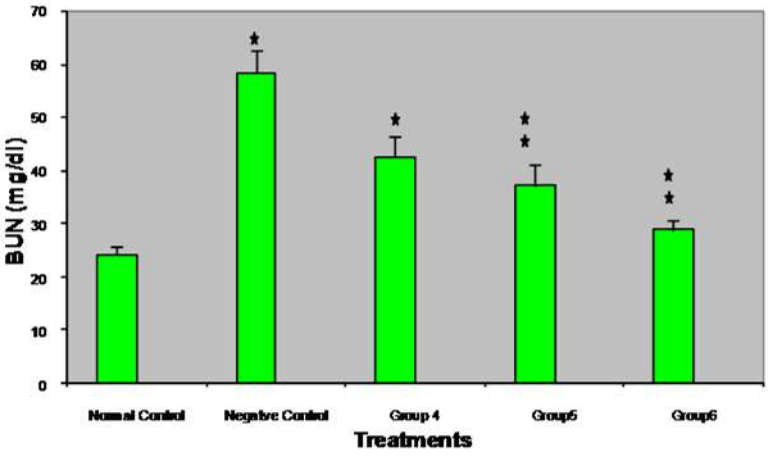

Fig. 2: Effect of aqueous extract F.*religiousa* (FR) on Blood Urea Nitrogen (BUN) level in mice treated with Cisplatin . Values are mean ± S.D, n = 6; *Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05). BUN: Blood Urea Nitrogen

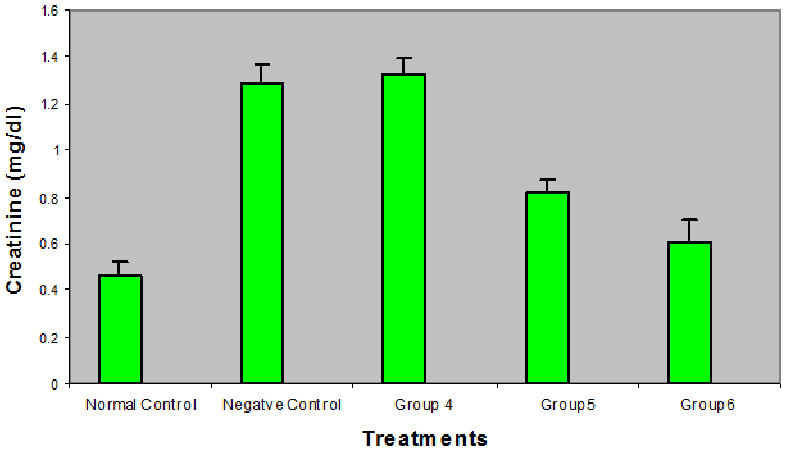

Fig. 3: Effect of aqueous extract F.*religiousa* (FR) on serum Creatinine level in mice treated with Cisplatin. Values are mean ± S.D, n = 6 *Indicate significance compared to control group (p< 0.05). **Indicate significance compared to Cis- group (p< 0.05).

CONCLUSION

Current study demonstrated the impairment of the antioxidant defense system and induced lipid peroxidation by Cisplatin. This study suggested the possible protective mechanism of FR by improving antioxidant status in mice which was induced nephro and hepatotoxicity. This effects was evidenced by the ability of this extract to return the reduced activities of intracellular antioxidants in liver and kidney. However, the FR antioxidant effect was dose dependent up to 1000 mg/ kg. The results of this study, suggested that supplementation of FR extract during the therapy period may play a protective role against side effects induced by Cisplatin.

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CITATION OF THIS ARTICLE
Farhad A and Varsha W. Ethanolic extract of *Ficus religiosa* prevents Cisplatin toxicity by Enhancing antioxidant status in Mice. Bull. Env. Pharmacol. Life Sci., Vol 3 [11] October 2014: 16-21