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**Experimental Studies of *Ficus religiosa* (L) latex for preventive and curative effect against cisplatin induced nephrotoxicity in wistar rats**

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**ABSTRACT**

The present study was designed to evaluate the possible potential, nephroprotective and nephrocurative role of 400mg/kg methanolic extract of *Ficus religiosa* L. latex was used against cisplatin (5mg/kg, i.p.) induced nephrotoxicity. The experimental protocol designed as the animals were divided into four groups (n=6) like control, model control, curative and protective groups were received vehicle, cisplatin, cisplatin + extract, and extract + cisplatin respectively. After 6<sup>th</sup> days, blood collected from retro-orbital sinus of rats and determined urea and creatinine level in serum of each group after then rats were sacrificed for quantitative estimation of various enzymes and ATPases content in kidney tissue. A single dose of cisplatin induced increased urea & creatinine level in serum it was significantly recovered by 400mg/kg in curative and protective groups. The enzyme estimation in kidney tissue it found that increased malondialdehyde and decreased reduced glutathione (GSH). It was significantly monitored by 400mg/kg in curative and protective groups. The level of brush border enzymes like Na<sup>+</sup> / K<sup>+</sup> ATPase, Ca<sup>++</sup> ATPase and Mg<sup>++</sup>ATPase were found significantly reduced after single dose cisplatin injection. It was overcome by treatment of same extract in curative and protective groups. Finally it is concluded that the present study data conformed nephrotoxicity induced by cisplatin due oxidative stress and methanolic extract of *Ficus religiosa* L. latex may have nephroprotective and curative activity.

**Keywords:** Cisplatin; Nephrotoxicity; urea; creatinine; glutathione; Lipid peroxidation.

**INTRODUCTION**

A large number of medicinal plants, natural products and dietary components have been evaluated as potential nephroprotective agents [1]. The *Ficus religiosa* (family-Moraceae) is

widely planted in the tropics [2]. The tree is very long lived and one tree near Bombay is reported to be over 3,000 years old [3]. The barks of *Ficus religiosa* species contains tannin, saponin gluanol acetate,  $\beta$ sitosterol, leucopelargonidin- 3 - O -  $\beta$  - D -glucopyranoside, leucopelargonidin - 3 - O -  $\alpha$  -L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate,  $\alpha$ -amyryn acetate, leucoanthocyanidin, and leucoanthocyanin [4]. Some reported pharmacological activity of *F. religiosa* like fruit extracts exhibited antitumor activity in the potato disc bioassay [5]. Aqueous extract was decreased the fasting blood glucose and exaggerated activity of superoxide dismutase SOD in streptozotocin induced type II diabetic rats [6], anthelmintic activity of the methenolic extract [7]. Aqueous extract showed high antimicrobial activity against selected pathogenic like *B. subtilis* and *P. aeruginosa* [8]. Cisplatin (cis-diamminedichloroplatinumII) (CDDP) is one of most potent anticancer drug. it is produced dose limiting nephrotoxicity and high dose of CDDP produce the impairment of kidney, causes decrease in renal blood flow, glomerular filtration rate and increases urea and creatinine level in blood [9]. The cisplatin induced nephrotoxicity was characterized by signs of injury such as changes in urine volume, body weight, increase the products of lipid peroxidation, and change renal clearance [10]. Kidneys have some antioxidant enzyme like superoxide dismutase (SOD), lipid peroxidase and glutathione (GSH), and Catalase which protect kidney from free radicals like nitric oxide and superoxide etc. The cisplatin is inhibited the activity of antioxidant enzyme in renal tissue like glutathione, SOD, GSH and Catalase depletion and increase thiobarbuturic acid - reactive substance (TBARS) [11]. Thus, the purpose of current study was to investigate whether oral administration of methanolic extract of *Ficus religiosa L.* latex has any protective and curative effect against cisplatin induced nephrotoxicity in albino rats.

## EXPERIMENTAL SECTION

### Drug and Reagents

Cisplatin (VHB, Life sciences Inc., India), DTNB (Merck Pvt. Ltd., India). Glutathione (Merck Pvt. Ltd., India), Thiobarbuturic acid (Loba chemicals pvt.ltd. India).

### Plant material

*Ficus religiosa L.* latex was collected from village Pipariya dist. Vadodara (G.P., India). The plant was identified by Dr. Nagar (Professor of botany), M.S. University Vadodara (Gujarat), and voucher specimen (DPSV/F/01/2010) was submitted in department of Pharmacy, Sumandeep Vidyapeeth, Pipariya Vadodara, Gujarat. *Ficus religiosa L* latex was extracted using methyl alcohol as a solvent. The extract was dried by rotator evaporator under reduced pressure.

### Animals

Adult male wistar rats having weight around 180-210 g were maintained at  $25 \pm 2^\circ\text{C}$  and kept in well ventilated animal house under photoperiodic condition in large polypropylene cages and were standard food and water *ad libitum*. The experiment was carried out in accordance to the guidelines mentioned in the CPCSEA, and Institutional Animal Ethical Committee approved the experiment protocols (Reg.No.-947/ac/06/CPCSEA).

### Experimental design

The acute toxicity study of methanolic extract of *Ficus religiosa L.* latex was not occurred at 2000mg/kg (as per the OECD - 420) on male Wistar rats The dose was selected one tenth ( $1/10^{\text{th}}$ ) and fifth ( $1/5^{\text{th}}$ ) of it, for safe treatment.

Total duration of study was 16 days. The animals were divided into four groups containing six animals in each group. Group I served as control and received normal saline throughout the experiment, Group II (Modal Control) received single dose of cisplatin (5mg/kg i.p.), 1<sup>st</sup> days, Group III (Protective) received extract (400mg / kg p.o.) for 1<sup>st</sup> to 10<sup>th</sup> day and 11<sup>th</sup> day, single dose (5mg/kg, i.p.) of cisplatin was administered, Group IV (Curative) received same dose of cisplatin on day 1<sup>st</sup>, and after 6<sup>th</sup> days extract (400mg / kg p.o.) was administered up to 16<sup>th</sup> days.

### **Biochemical assays**

After the treatment period, blood was collected from retro-orbital sinus of rat under ether anaesthesia and centrifuged using the table top centrifuge (REMI) at 3000 rpm to get serum. Level of urea and creatinine was estimated using Span diagnostic kit on chemical analyzer (microlab3000) for assessment of renal toxicity. [12&13], After then Kidneys were removed, homogenized and centrifuged at 10,000 rpm at 0°C for 20 min. the supernatant was used for estimation of different antioxidant level by calorimetric method using spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam), Glutathione reductase (GSH) estimated by Sedlak and Lindsay method [14 & 15], Lipid peroxidation by thiobarbuturic acid-reactive substances (TBARS) methods [16, 17&18], and the sediment of the centrifuge was used for estimation of the Na<sup>+</sup>K<sup>+</sup>ATPase by Bontin methods [19], Ca<sup>2+</sup>ATPase by Hjerken and Pan [20], Mg<sup>2+</sup>ATPase by Ohinishi *et al.* method [21].

### **Statistical analysis**

Results were expressed as one way analysis of variance (ANOVA) followed by Dunnett's test and P < 0.05 was considered as significant.

## **RESULTS AND DISCUSSION**

There are a large number of synthetic drugs induce nephrotoxicity and acute renal failure. The acute renal failure is reversible loss of renal functions. It may be recovered by herbal medicine. In kidney having some naturally occurring antioxidant enzyme like Superoxide dismutase (SOD), lipid peroxidase, catalase and glutathione (GSH), which protect kidney from free radicals, induce oxidative renal impairment.

After injected the single dose of cisplatin (5mg/kg) causes increased urea (76.66±2.24) and creatinine (2.32±0.10) level in model control compare to respective control group (24.16±1.04 and 0.94±0.05) and its was recovered significantly (\*\*P<0.01) in curative and protective groups with treatment of 400mg/kg same extract (Table no.1). The change of renal function observed in the rat correlate well with the nephrotoxicity effect with man [22]. The increased urea and creatinine level suggests the reduction of glomerular filtration rate [23]. The present study was revealed that significantly decrease the level of urea and creatinine in blood serum after treatment of methanolic extract of *Ficus religiosa* L. latex that indicates increase glomerular filtration rate.

Jeong *et al* [24] observed that a single injection of cisplatin dose 5mg/kg body weight in rabbit caused a mark reduction of glomerular filtration rate, which is accompanied by increase in serum creatinine level indicating induction of acute renal failure. According to previous findings, we conformed that a single dose cisplatin induced a significantly serum creatinine in wistar rats three to seven days after administration [25, 26]. The present study data had shown that significantly recovery of urea and creatinine serum level in curative and protective groups with treatment of both 400mg/kg same extract.

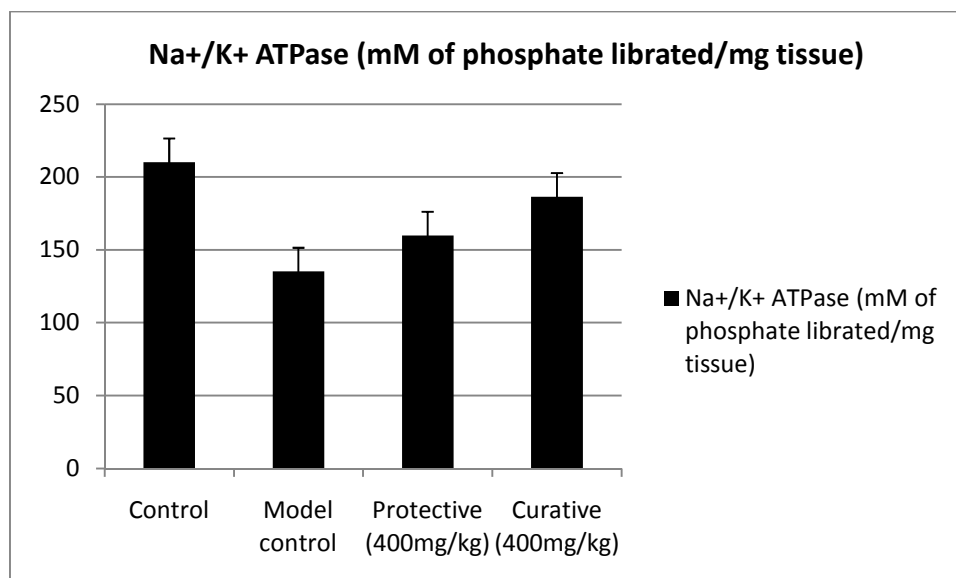
In aspect of kidney tissue estimation, it is shown as significantly (\*\*P<0.01) increase the lipid peroxidase (24.50±0.61) and decrease the level of GSH (45.33±1.66) after single dose injection of cisplatin in model control group. The lipid peroxidase, GSH, level were significantly (\*\*P<0.01) monitored with dose 400mg/kg in curative and protective groups (Table no.1). After a single dose of cisplatin injection decreased GSH, and increased lipid peroxidation level in model control that indicates production of free radicals involvement of oxidative stress due to cisplatin induced nephrotoxicity [27]. Our present result data shown that significantly monitored GSH and lipid peroxidation. This is indicate that extract have antioxidant activity. Whereas in present phytochemical study of the extract have revealed the presence of Flavonoids, amino acids and tannin (Phenolic compound), Flavonoids have antioxidant activity and amino acid were used to synthesis of the endogenous glutathione. It's all may contribute synergistic reason to increase GSH level in kidney tissue significantly.

**Table no.1. Effect of methanolic extract of *Ficus religiosa* L latex on urea, and creatinine in blood serum, GSH and lipid peroxidation in Kidney tissue**

S.No.	Groups	Urea level in serum (mg/dl)	Creatinine level in serum(mg/dl)	µmol GSH/gm. Kidney tissue	n Mol MDA/gm. MI
1.	Control	24.16±1.04	0.94±0.05	69.50±1.54	14.00±0.57
2.	Model control	76.66±2.24 <sup>a</sup>	2.32±0.10 <sup>a</sup>	45.33±1.66 <sup>a</sup>	24.50±0.61 <sup>a</sup>
3.	Protective(400mg/kg)	52.33±0.91 <sup>b</sup>	1.56±0.09 <sup>b</sup>	58.50±1.40 <sup>b</sup>	17.66±0.61 <sup>b</sup>
4.	Curative (400mg/kg)	31.16±0.70 <sup>b</sup>	1.15±0.15 <sup>b</sup>	63.83±1.19 <sup>b</sup>	15.50±0.56 <sup>b</sup>

<sup>a</sup>\*\*P<0.01 as compared to the Control

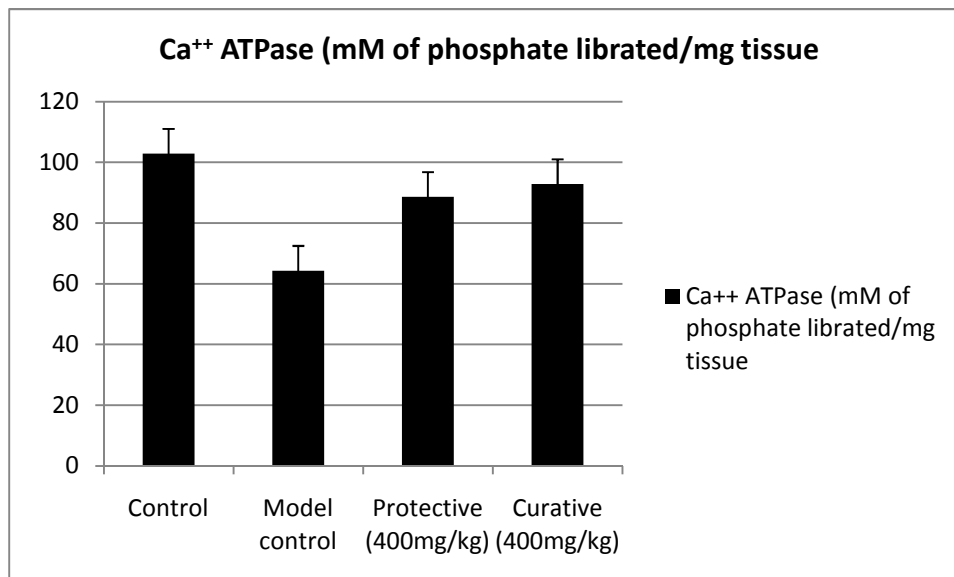
<sup>b</sup>\*\*P<0.01 as compared to the model Control



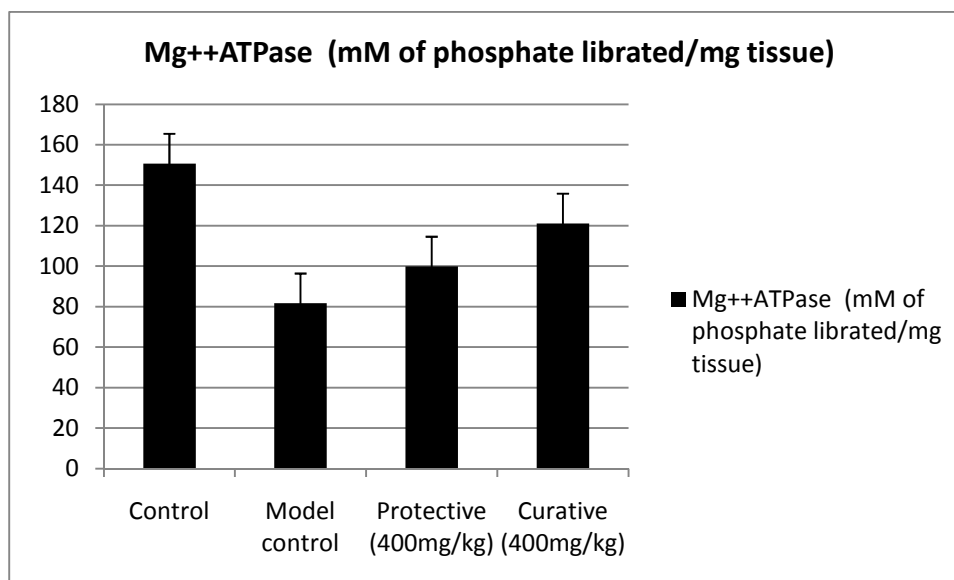
**Figure1. Effect of methanolic extract of *Ficus religiosa* L latex on Na<sup>+</sup>/K<sup>+</sup> ATPase of various groups. Each group represents mean±S.D. of six animals, \*\*P<0.01, \*\*P>0.01, \*\*P>0.01, as compared to the Model Control.**

It's all may contribute synergistic reason to increase GSH level in kidney tissue significantly. The level of brush border enzymes like Na<sup>+</sup>/K<sup>+</sup> ATPase, Ca<sup>++</sup> ATPase and Mg<sup>++</sup>ATPase were found to reduced significantly (\*\*P<0.01) in model control group as compared with control group, the Na<sup>+</sup>/K<sup>+</sup> ATPase, Mg<sup>++</sup>ATPase and The Mg<sup>++</sup>ATPase were Significantly (\*\*P<0.01) recovered with dose 400mg/kg in protective and curative groups (fig.1,2,&3). After damage of kidney, pathophysiological change in occur in proximal tubules cisplatin

toxicity by formation of reactive species which cause the redistribution of brush border enzyme [28]. In present result data reveal that the level of membrane bound enzyme  $\text{Na}^+/\text{K}^+$  ATPase,  $\text{Ca}^{++}$  ATPase and  $\text{Mg}^{++}$ ATPase were significantly decrease after cisplatin administration whereas it overcome significantly in extract treated groups. It is indicates that extract may have capacity to deduced cisplatin induced nephrotoxicity.



**Figure2. Effect of methanolic extract of *Ficus religiosa* L latex on  $\text{Ca}^{++}$  ATPase of various groups.** Each group represents mean  $\pm$  S.D. of six animals,  $**P < 0.01$ ,  $**P > 0.01$ ,  $**P > 0.01$ , as compared to the Model Control.



**Figure3. Effect of methanolic extract of *Ficus religiosa* L latex on  $\text{Mg}^{++}$  ATPase of various groups.** Each group represents mean  $\pm$  S.D. of six animals,  $**P < 0.01$ ,  $**P > 0.01$ ,  $**P > 0.01$ , as compared to the Model Control.

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## CONCLUSION

Finally it is concluded that the present study data conformed nephrotoxicity induced by cisplatin due oxidative stress and methanolic extract of *Ficus religiosa* L. latex may have nephroprotective and nephrocurative.

## REFERENCES

- [1]. Bergstrom J, Furst P, Norée LO, Vinnars E. *J Appl Physiol.* **1974**; 36; 693–6.
- [2]. Bailey, L. H. and E. Z. Bailey. *Hortus*. 3rd ed. Macmillan General Reference, NY. **1976**.
- [3] Neal, M.C... *In Gardens of Hawai'i*. Bernice P. Bishop Museum Special Publication 40, Bishop Museum Press, Honolulu, HI; **1965**.
- [4]. Husain A, Virmani OP, Popli SP, Misra LN, Gupta MM, Srivastava GN, Abraham Z & Singh AK. Dictionary of Indian Medicinal Plants, CIMAP, Lucknow, India. **1992**; 546
- [5]. Mousa O, Vuorela P, Kiviranta J, Wahab SA, Hiltohen R, Vuorela H *J Ethnopharmacol*: **1994**; 41:71- 6,
- [6]. Kirana H. ,SS Agarwal, Srinivasan B P., *Indian Journal of Experimental biology.* **2009**; 47: pp 822-826,
- [7]. Zafar Iqbal, Qazi khalid Nadeem, M.N. Khan, M.S Akhtar and Faisal Nnouman Waraich *International journal of agriculture & biology.* **2001**;3(4):454–457.2
- [8]. Preethi R., Vimal V. Devanathan and Loganathan M., *Advances in Biological Research.* **2010**; 4 (2): 122-125.
- [9] Gonzalez Ricardo, Romay Cheyla, Borrego A, Merino FH, Zamora MZ, and Rojas E *Inflamm.* **2005**; 3: 139-143.
- [10] Kersten L, Braunlich H, Kepper BK, Gliesing C, Wendelin M, Westphal J. *J appl toxicol.* **1998**; 8(2): 245
- [11] Zhang JG, Lindup WE. *Biochem pharmacol.* **1993**;45 (11): 677 – 683.
- [12] Kaplan A, Lavamen IS. The tests of renal function, in clinical chemistry. Interpretation and techniques 2<sup>nd</sup> ed., Lea and Febiger, Philadelphia. **1983**; 109-142.
- [13] Varley, and Alan HG. Tests in renal disease. In Practical Clinical Biochemistry. **1984**; 1: 123.
- [14] Sedlak M, and Lindsay R. *Anal Biochem.* **1968**; 25:192–205.
- [15] Hartree E. *Anal Biochem*, **1972**; 48: 4227.
- [16] Zager RA. *Kidney Int.* **1998**; 33: 84-90.
- [17] Didier Portilla, Richard TP, Safar AM. Cisplatin-induced nephrotoxicity Last literature review version. **2008**; 16.3.
- [18] Uchiyama M, Mihara M. *Anal Biochem.* **1978**; 86: 271–8.
- [19]. Bontin SL, Presence of enzyme system of mammalian tissue. Wiley inter sci. **1970**: 197,257.
- [20]. Hjerken S, Pan H. *Biochem Biophys Acta*, (**1983**); 728: 281.
- [21]. Ohinishi T, Suzuki Y, Ozawa K. *Biochem biophys ACTA*, **1982**; 684: 64.
- [22]. Daugaard G, Abilgaard U, Holstein-Rathlou NH, Bruunshuus I, Bucher D, Leyssac PP. *Clin Pharmacol Ther.* **1988**; 44:164–72.
- [23]. Naziroglu M, Karaoglu A, Aksoy AO, *Toxicolo.* **2004**; 195: 221-239.
- [24]. Jeong JC, Hwang WM, Yoon CH, Kim YK, Salviae radix prevent cisplatin induced acute renal failure, nephron; 2001; 88;241-6
- [25]. Francescato HDC, Costa RS, Camargo SMR, Zanetti MA, Lavrador MA, Bianchi MLP. *Pharmacol Res* **2001**; 43:77–82.
- [26]. Attunes LMG, Darin JDC, and Bianchi MLP. *Pharmacol Res* **2001**; 43:145–50.

[27]. Nelson DL, and Michael M C. Lehninger principle of biochemistry. 4<sup>th</sup> edition; **2005**; 857-858.

[28]. Molitoris BA, Dahl R, Geerdes A. *Am J. Physical*, **1992**; 265,F488-F495.