Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

# Nephroprotective Effects of *Ficus religiosa Linn* (Peepal Plant) Stem Bark against Isoniazid and Rifampicin Induced nephrotoxicity in Albino Rabbits

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ARTICLE HISTORY

Received: December 29, 2012
Revised: February 23, 2013
Accepted: April 17, 2013

**Key words:**
HPLC
Isoniazid
Nephroprotection
Rifampicin
Toxicity

**ABSTRACT**

Tuberculosis (TB) is an airborne infection that requires at least six to nine months treatment. Long term treatment of TB may produce severe toxic effects to organs such as kidney, liver, gastrointestinal and blood vascular systems. The present study was designed to evaluate the nephroprotective effects of *Ficus religiosa* (peepal plant) stem bark against the toxic effects induced by anti TB drugs rifampicin (RIF) and isoniazid (INH). For this purpose, 40 rabbits were divided into five groups (n=8). Group 1 was kept as control without drugs. Group 2 was given anti TB drugs orally for 28 days while groups 3, 4 and 5 were given silymarin, ethanolic ficus extract, and water ficus extract along with anti TB drugs orally for 28 days respectively. Blood samples were taken before drug administration and at 7$^{th}$, 14$^{th}$, 21$^{st}$, and 28$^{th}$ days post treatment for biochemical and drug analysis. Kidney tissues were taken for histopathological studies. Results have indicated that Mean±SEM blood urea nitrogen (BUN) was increased significantly ($P<0.05$) in RIF + INH-treated rabbits (45.94±2.15) as compared to control group (27.86±0.22). The administration of silymarin reduced BUN (33.17±1.87) towards normal. The administration of alcohol extract of *F. religiosa* further reduced this BUN level (32.15±1.67), almost close to normal value while its hydro extract also reduced the BUN level (34.85±2.85) significantly but less than alcoholic extract of *F. religiosa* and silymarin. Similar findings were found with creatinine. Kidney of RIF + INH-treated rabbits showed severe degree of infiltration in the glomerulas without renal tubular space between the glomerulas when compared to normal group. Drug analysis with HPLC revealed that drug concentration in plasma coincides with the toxicity of RIF and INH. In conclusion, the alcoholic as well as water extracts of *F. religiosa* have shown potent nephroprotective effects when administered along with the RIF+INH and have shown results similar to that of silymarin.

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**To Cite This Article:** Hashmi N, F Muhammad, I Javed, JA Khan, MZ Khan, T Khaliq and B Aslam, 2013. Nephroprotective effects of *Ficus religiosa linn* (peepal plant) stem bark against isoniazid and rifampicin induced nephrotoxicity in albino rabbits. Pak Vet J, 33(3): 330-334.

## INTRODUCTION

Tuberculosis (TB) is the worldwide fatal disease that can spread amongst the people easily. It is estimated that 1/3$^{rd}$ population of the world is assumed to be infected with pathogen (*Myobacterium tuberculosis*) but two million people die of this disease every year (Shishoo *et al*., 2001). TB has been declared by World health organization (WHO) as global health emergency (Anonymous, 1997).

Standard treatment for TB is combination of three drugs rifampicin, isoniazid and pyrazinamide used for at least eight weeks then continue the therapy with isoniazid and rifampicin for 4-7 months. Rifampicin has been reported to have some adverse effects including nephrotoxicity (Haptinstall, 1976; Hirsch *et al*., 1983). The most common form of nephrotoxicity is a syndrome of acute renal failure, with tubular necrosis, with or without hemolysis and thrombocytopenia, commonly occurring during intermittent or interrupted treatment. Other types of nephrotoxicity are interstitial nephritis with or without mild glomerular lesions, rapidly progressive glomerulonephritis and light chain proteinuria (De Vriese *et al*., 1998).

Now-a-days medicinal plants are widely used to cure many diseases owing their cheapness, safety and non-toxicity in comparison to synthetic drugs (Bhawna and Kumar, 2009). *F. religiosa*, is a very common tree in Pakistan, with heart shaped long tipped green leaves and grey brown bark. The plant has phytochemical constituents that include tannins, saponins, flavonoids, and cardiac glycosides. It is native to Bengal, Central India and Sub–Himalayan tract (McFarland, 1944).

In traditional medicine *F. religiosa* has been used as carminatives, astringent, vermonicides, hypotensive, antihelminthics, stomachics and anti dysentery drugs (Trivedi *et al*., 1969). With respect to traditional medicinal uses of different parts of *F. religiosa*, bark is used for inflammation, diarrhea and burns and as antibacterial agent, leaves for wounds and skin diseases, seeds as laxative, latex for inflammation (Warrier, 1996), leaf juice for asthma, migraine and cough (Warrier, 1996; Kunwar and Bussmann, 2006) and dried fruit for TB (Khanom *et al*., 2000).

In spite of the vast pharmacological activities of this plant, its protective effects against nephrotoxicity associated with the use of antituberculosis drugs have not been documented. Hence, the present study was designed to evaluate the nephroprotective effects of this plant extract against antituberculosis drugs (Isoniazid and Rifampicin).

## MATERIALS AND METHODS

**Chemicals:** Pure INH (99%) and RIF (99%) were gifted by Pacific Pharmaceuticals Ltd, Lahore, Pakistan and 99% pure Silymarin was taken from Abbott laboratories, Karachi, Pakistan. All reagents used were of analytical grade.

**Plant materials:** *F. religiosa* stem bark was obtained from the vicinity of University of Agriculture Faisalabad and was authenticated by a botanist at the Department of Botany, University of Agriculture Faisalabad. Bark stem was shade dried and powdered with the help of mechanical grinder. Powder was passed through mesh sieve and placed in airtight container. Water extract was prepared by macerating the powdered stem bark of *F. religiosa* in distilled water at room temperature for 2 days and filtered with Whatman filter paper (Pandit *et al*., 2010). Ethanolic extract was prepared from the stem bark of *F. religiosa* using Soxhlet apparatus (Mounnissamy *et al*., 2010).

**Experimental protocol:** Adult albino rabbits of age (8-10 months) and weight (1300-1500 gram) were purchased from the local market in Faisalabad-Pakistan and kept at room temperature at the Department of Physiology and Pharmacology, University of Agriculture Faisalabad-Pakistan. Seasonal fodder and water were offered *ad libitum*. Forty rabbits were divided in five groups each having 8 rabbits and they were treated following the protocol as shown in Table 1.

**Biochemical analysis:** Kidney functions were assessed by blood urea nitrogen (BUN), level of creatinine in serum using commercially available kits (Randox, Admore, Diamond, Road, Crumlin, CO., Antrim United Kingdom).

**Histopathological analysis:** At the end of experimental procedure, rabbits were euthanized with ketamine and kidney were excised and fixed in formaline and further biopsies were processed through graded concentrations of ethanol and embedded in paraffin blocks. The sections of kidney were oriented perpendicular to the plane of section in the block and 6 micrometer thick transverse sections were cut and mounted on glass slides and stained with hematoxylin and eosin. Histological examination of slides was made by Olympus PM – 10ADS automatic light microscope (Olympus optical Co., Tokyo, Japan) with a 400X objective.

**Plasma drug analysis:** Before and after 1, 2, 3, and 4 weeks of drug administration, blood was taken in heparinized tubes in order to determine the drug concentration in plasma with HPLC. The drug analysis of INH+RIF was performed with HPLC (Schimadzu, Germany) at central Hi-Tech laboratory, University of Agriculture Faisalabad-Pakistan. The chromatographic conditions of INH were as follows, column Shim- pack CLC-ODS (C-18), 15cm x 4.6mm, 5µm, mobile phase was isocratic (water, acetonitrile, triethylamine and acetic acid in ration of 600:400:2:1), flow rate was 1ml/min and detection UV was 333nm. The chromatographic conditions of RIF were as follows, column Shim- pack CLC-ODS (C-18), 15cm x 4.6mm, 5µm, mobile phase was isocratic (water and acetonitrile in ratio of 40:60 V/V), flow rate was 1ml/min and detection UV was 333nm.

**Statistical analysis:** The results were expressed as mean±SEM. Statistical analysis was conducted by one way analysis of variance (ANOVA) (Steel *et al*., 1997) followed by LSD (SPSS version 16) at 5% level of significance ($P<0.05$).

## RESULTS AND DISCUSSION

Mean±SEM BUN level (mg/dl) was increased significantly ($P<0.05$) in RIF + INH treated rabbits (45.94±2.15) as compared to control group (27.86±0.22). The administration of silymarin reduced BUN level (33.17±1.87) towards normal. The administration of alcoholic extract of *F. religiosa* further reduced this BUN level (32.15±1.67), almost close to normal value, while its hydro extract also reduced the BUN level (34.85±2.85) significantly but less than alcoholic extract of *F. religiosa* and silymarin (Fig. 1).

Mean±SEM Creatinine level (mg/dl) was increased in RIF + INH treated rabbits (1.004±0.040) as compared to control group (0.742±0.037). The administration of silymarin reduced Creatinine level (0.872±0.044) towards normal. The administration of alcoholic extract of *F. religiosa* further reduced this Creatinine level (0.816.±0.033) significantly, almost close to normal value, while its hydro extract could not reduce this Creatinine level (0.938±0.026) significantly towards normal value but less than alcoholic extract of *F. religiosa* and silymarin (Fig. 2).

**Table 1:** Feeding and drugs administration schedule in albino rabbits during the experimental period of 0 to 28 days

| | |
|---|---|
| Group 1: (n=8) | Control animals on routine diet for 28 days. |
| Group 2: (n=8) | Routine diet + Isoniazid (50mg/kg b.w.) + Rifampicin (250mg/kg b.w.) P.O. daily for 28 days as nephrototoxic drugs. |
| Group 3: (n=8) | Routine diet + Isoniazid (50mg/kg body weight b.w.) + Rifampicin (250 mg/kg body weight b.w.) as nephrotoxic drugs along with Silymarin (2.5mg/kg b.w.) as nephroprotective drug P.O. daily for 28 days |
| Group 4: (n=8) | Routine diet + Isoniazid (50mg/kg body weight b.w.) + Rifampicin (250 mg/kg body weight b.w.) as nephrotoxic drugs along with aqueous bark extract of *F. religiosa* (250mg/kg b.w.) P.O. daily for 28 days. |
| Group 5: (n=8) | Routine diet + Isoniazid (50mg/kg body weight b.w.) + Rifampicin (250 mg/kg body weight b.w.) as nephrotoxic drugs along with ethanolic bark extract of *F. religiosa* (250mg/kg b.w.) P.O. daily for 28 days. |

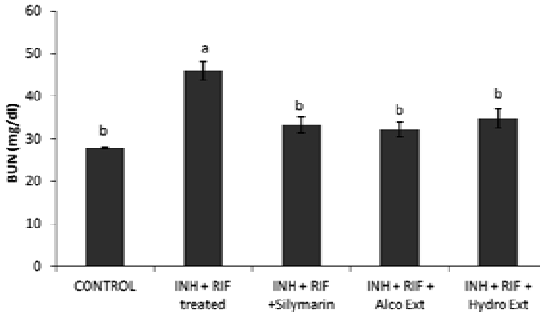


**Fig. 1:** Mean ±SEM serum BUN level (mg/dl) in control and treated groups with per oral drugs and *F. religiosa* extracts in rabbits (n=8) for 28 days. Columns bearing same alphabets are not statistically significant (P <0.05).

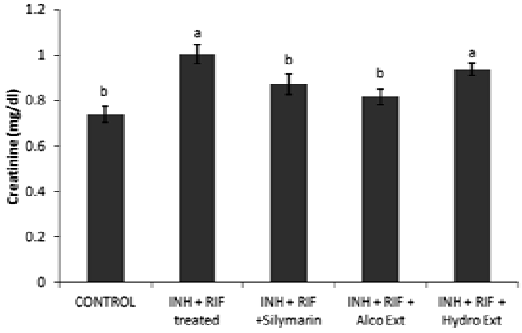


**Fig 2:** Mean±SEM serum CREATININE level (mg/dl) in control and treated groups with per oral drugs and *F. religiosa* extracts in rabbits (n=8) for 28 days. Columns bearing same alphabets are not statistically significant (P<0.05).

Both drugs (RIF + INH) induced significant renal damage to rabbits in this study. This observation is in accordance with Renugadevi and Prabu (2009), who observed significant renal damage as evidenced from the increased level of serum urea and creatinine after oral administration of cadmium (5 mg/kg day) in rats for four weeks. The alcoholic as well as aqueous extract of *F. religiosa* successfully reduced renal damage by significantly reducing the BUN (Fig. 1) and creatinine level (Fig. 2). The higher protective potential of alcoholic extract as compared to water extract might be due to its higher concentration of active constituents of plant responsible for nephroprotective action. This evidence again is in compliance with the study of Renugadevi and Prabu (2009), who reported that co-administraion of naringenin (25 and 50 mg/(kg day) along with cadmiun (5 mg/kg day) resulted in a reversal of cadmium induced biochemical changes in kidney. Similar observations were reported by Khan and Siddique (2012), who observed that *Citharexylum spinosum* and silymarin produced significant protective effects by restoring the concentration of serum markers such as urea, creatinine, creatinine clearance, albumin and protein.

In present studies, the histopathological findings of kidney of control group indicated that in the proximal convoluted tubules nuclei were normal in appearance, renal parenchyma was also normal in structure. Kidney of RIF + INH treated rabbits showed severe degree of infiltration in the glomerulus with-out renal tubular space between the glomerulus. There was mild to moderate congestion in the renal parenchyma, mild to moderate necrosis and nucleus are condensed in appearance as compared to control group (Fig. 3).

Renal tubular cells of RIF+INH+ silymarin treated rabbits showed mild degree of congestion, mild degree of necrosis, normal appearance of nuclei that showed protective effect of silymarin. Concomitant administration of ethanolic extract of *F. religiosa* prevented RIF+INH induced histopathological injuries in the renal tubular cells. This was evident from the normal appearance of the nuclei with no condensed nucleus. There was no necrosis, but at some places there was mild congestion (Fig. 4). Kidney tubular cells structure was normal in appearance that showed protective effect of ethanolic extract of *F. religiosa* as compared to INH+RIF treated groups. While aqueous extract treated group showed mild degree of congestion, mild degree of necrosis, at few places nucleus was condensed while at other places nucleus was normal in appearance that indicated protective effect of aqueous extract of *F. religiosa*.

Nephroprotective effects of other plants have also been reported such as naringenin (50 mg/kg/day) decreased the toxicity of cadmium and preserved the normal histological structure of the renal tissue (Renugadevi and Prabu, 2009) and *Allium ascalonicum* provided protective effects against cyclosporine induced renal damage (Wongmekiat *et al*., 2008). These results are correlated well with the earlier studies demonstrating significant renal dysfunction in patients and in the experimental animals following cyclosporine A administration (Tariq *et al*., 1999; Anjaneyulu *et al*., 2003; Burdmann *et al*., 2003; Wongmekiat and Thamprasert, 2005). Based on these studies, this might be postulated that *F. religiosa* extracts might contain active constituents responsible for nephroprotective potential. This plant has phytochemical constituents such as tannins, saponins, flavonoids, and glycosides (Warrier, 1996; Kunwar *et al*., 2006) that might be responsible for nephroprotective activity also.

The relationship of plasma drug concentration along with the biochemical and histopathological findings in drug induced nephrotoxicity has not been reported yet, so our study is first of its kind to observe this relationship. Drug analysis with HPLC method indicated that INH and RIF concentrations were higher at 4$^{th}$ week as compared

**Table 2:** High Performance Liquid Chromatography (HPLC) analysis of Isoniazid and Rifampicin

| Groups | INH concentration at 2nd Week (µg/ml) | INH concentration at 4th Week ( µg/ml) | RIF concentration at 2nd Week (µg/ml) | RIF concentration at 4th Week (µg/ml) |
|---|---|---|---|---|
| RIF+INH | 17.25 | 35.40 | 21.27 | 40.97 |
| RIF+INH+Silymarin | 13.66 | 17.26 | 15.69 | 20.13 |
| RIF+INH+Alcoholic extract of *F.religiosa* | 25.31 | 12.76 | 42.53 | 27.89 |
| RIF+INH +Water extract of *F.religiosa* | 8.93 | 10.38 | 7.50 | 10.56 |

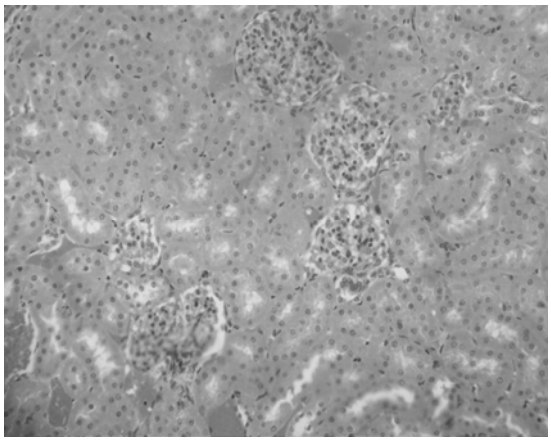

**Fig. 3:** Kidney of rabbit treated with INH 50 mg/kg per body weight + RIF 250 mg/kg per body weight with daily oral administration for 28 days (H & E, 400x).

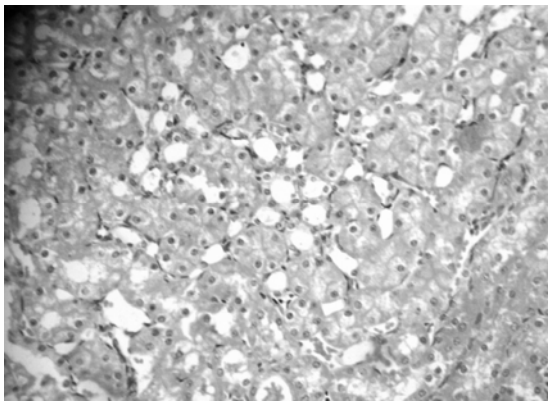

**Fig. 4:** Kidney of rabbit treated with RIF 250 mg/kg body weight + INH 50mg/kg body weight + ethanolic extract of *F. religiosa* 250mg/kg body weight with daily oral administration for 28 days (H & E, 400x).

to 2nd week in RIF + INH treated group (Table 2). This is probably due to accumulation of drug in the body over the period of time. Since the half life of RIF is 1.5-5 hours and INH is 1-4 hours (Brunton *et al*., 2006), so after 24 hours, the considerable amount of drug may be retained in the body tissues and can cause toxicity in the target organs especially kidney. This observation is confirmed with the increased level of BUN and creatinine in this treated group in our study. Almost the same trend of drug concentration was observed in silymarin treated group but with less concentration. This was probably due to the protective effect of silymarin on these drug induced toxicity as evidenced by reduced value of biochemical parameters. In group that has been co-administered with alcoholic extract of *F. religiosa* along with the RIF+INH, both drugs concentration (RIF+INH) was higher at 2nd week that was decreased at 4th week of drug administration. This was probably due to the effect of plant extract on diuresis (Joseph and Raj, 2010), therefore, this can be postulated that this plant extract has increased the urinary excretion of these drugs thus reducing the concentration of the drugs at 4th week. This observation is supported by the biochemical and histopathological changes in this group of animals as BUN and creatinine were significantly reduced and microscopic picture of nephron was improved as compared to the control. Similar observations were observed with water extract of the plant but with less frequency as compared to the alcoholic extract of the plant.

**Conclusion:** It was concluded that RIF+INH cause nephrotoxicity in rabbits. The present study has shown that if some nephroprotective agents such as silymarin administered along with the RIF+INH then the toxic effects of these drugs can be minimized. The alcoholic as well as water extracts of *F. religiosa* stem bark have shown potent nephroprotective effects when administered along with the RIF+INH and have shown results similar to that of silymarin. In this study, alcoholic extract was found to be more effective as compared to water extract.

## REFERENCES


Anjaneyulu M, N Tirkey and K Chopra, 2003. Attenuation of cyclosporine-induced renal dysfunction by catechin: possible antioxidant mechanism. Renal Fail, 25: 691-707.

Anonymous X, 1997. The resurgence of tuberculosis: a call for commitment. General policy topics WHO. Drug Info, 5: 39-40.

Bhawna S and SU Kumar, 2009. Hepatoprotective activity of some indigenous plants. Int J Pharm Tech, 1: 1330-1334.

Brunton LL, JS Lazo and KL Parker, 2006. Goodman and Gillman's Pharmacological Basis of therapeutics. 11th Ed., Mc Graw Hill, New York, USA.

Burdmann EA, TF Andoh, L Yu and WM Bennett, 2003. Cyclosporine nephrotoxicity. Semin Nephrol, 23: 465-476.

De Vriese AS, DL Robbrecht, RC Vanholder, DP Vogelaers and NH Lameire, 1998. Rifampicin- associated acute renal failure: Pathophysiologic, immunologic and clinical features. Am J Kidney Dis, 31: 108-115.

Haptinstall RH, 1976. Interstitial nephritis. Am J Pathol, 83: 214-236.

Hirsch DJ, FJ Bia, M Kashgarian and MJ Bia, 1983. Rapidly progressive glomerulonephritis during antituberculous therapy. Am J Nephrol, 3: 7-10.

Joseph B and SJ Raj, 2010. Phytopharmacological and phytochemical properties of three ficus species - an overview. Int J Pharma Bio Sci, 1: 246-253.

Khan MR and F Siddique, 2012. Antioxidant effects of *Citharexylum spinosum* in CCl4 induced nephrotoxicity in rat. Exp Toxicol Pathol, 64: 349-355.

Khanom F, H Kayahara and K Tadasa, 2000. Biosci Biotechnol Biochem, 64: 837-840.

Kunwar RM and RW Bussmann, 2006. *Ficus* (fig) species in Nepal: a review of diversity and indigenous uses. Lyonia-J Ecol Appl, 11: 85-97.

McFarland GB, 1944. Thai-English Dictionary. Stanford University press, Stanford, California, USA pp: 601.

Mounnissamy VM, S Kavimani, G Sankari, SD Quine and K Subramani, 2010. Evaluation of acute and sub-acute toxicity of ethanol extracts of *Cansjerarheedi* J Gmelin (opiliaceae). J Brewing Distilling, 1: 11-14.

Pandit R, A Phadke and A Jagtap, 2010. Antidiabetic effect of *Ficus religiosa* extract in streptozotocin induced diabetic rats. J Ethnopharm, 128: 462-466.

Renugadevi J and SM Prabu, 2009. Naringenin protects against cadmium-induced oxidative renal dysfunction in rats. Toxicology 256: 128-134.

Shishoo CJ, SA Shar, IS Rathod, SS Savale and MJ Vora, 2001. Impaired bioavailability of rifampicin in presence of isoniazid from fixed dose combination (FDC) formulation. Int J Pharm, 228: 53-67.

Steel RGD, JH Torrie and DA Dieky, 1997. Principles and Procedures of Statistics. 3rd Ed, McGraw-Hill Book Co Inc, New York, USA.

Tariq M, C Morais, S Sobki, M Al Sulaiman and A Al Khader, 1999. N-acetylcysteine attenuates cyclosporine-induced nephrotoxicity in rats. Nephrol Dial Transp, 14: 923-929.

Trivedi P, S Hinde and RC Sharma, 1969. Preliminary phytochemical and pharmacological studies on *Ficus racemosa*. J Med Res, 56: 1070-1074.

Warrier PK, 1996. Indian medicinal plants-A compendium of 500 species, Orient Longman Ltd, Chennai, 3**:** 38-39.

Wongmekiat O and K Thamprasert, 2005. Investigating the protective effects of aged garlic extract on cyclosporine-induced nephrotoxicity in rats. Fundam Clin Pharmacol 19: 555-562.

Wongmekiat O, N Leelarugrayub and K Thamprasert, 2008. Beneficial effects of shallot (*Allium ascalonicum* L.) extract on cyclosporine nephrotoxicity in rats. Food Chem Toxicol, 46: 1844-1850.