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ORIGNAL ARTICLE

Nephroprotective effects of *Raphanus sativus* (Radish) in rifampicin induced nephrotoxicity in adult albino rabbits

Rehan Khalid[1*], Muhammad Irfan Anwar[2] and Aisha Ambreen[3]

[1]*Faisalabad Institute of Health Sciences;* [2]*Department of Pathobiology, Bahauddin Zakariya University Multan;*
[3]*Department of Biochemistry, Faisalabad Medical University Faisalabad*

Abstract

Rifampicin is one of the broad-spectrum antibiotics and it is a key component of antituberculosis treatment. Its adverse effects include acute renal failure and nephrotoxicity. Silymarin is being used to counter the adverse effects of rifampicin as it antagonizes the nephrotoxic effects of rifampicin due to its antioxidant and permeability stabilizing activity and hence prevents the entry of toxic agent in the cell. The leaves and stem part of *Raphanus sativus* also possesses strong antioxidant activity which make it a potent nephroprotective agent for nephrotoxicity induced by rifampicin. The current study was designed to assess the nephroprotective effects of *Raphanus sativus* (radish) leaves against nephrotoxicity induced by rifampicin. There were forty rabbits used and distributed in five different groups; Group-1 was control (CK). Rifampicin orally for 28 days was given to Group-2. For Group-3, 4 and 5, silymarin, ethanolic and aqueous *Raphanus sativus* extracts were administered orally, together with rifampicin, for 28 days, respectively. Blood samples were collected prior to the medication and at 7th, 14th, 21st, and 28th days after treatment for biochemical analysis. Renal tissue was collected for histopathological studies. Results showed that the rifampicin-treated group showed nephrotoxicity which increases blood urea nitrogen, serum creatinine and blood urea. Whereas groups treated with *Raphanus sativus* leaves aqueous and ethanol extracts along with rifampicin showed decrease in RFT levels due to their nephroprotective properties. Histopathological results showed congestion of renal tubules and glomeruli, which was the indication of toxicity associated with the use of rifampicin. The microstructures were returning to normal with the administration of nephroprotective drugs. Finally, alcoholic and aqueous extracts of *Raphanus sativus* when given along with rifampicin and have shown potent nephro-protective effects and shown results like that of silymarin.

Keywords

Nephroprotective
Raphanus sativus
Rifampicin
Silymarin
Tuberculosis

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Introduction

Tuberculosis is a communicable disease and spreads among the population. Millions of people are infected by tuberculosis every year worldwide. The occurrence of tuberculosis is very large in Pakistan. The total number of cases which was reported in 2008 was 286,000 (Javaid A *et al*., 2008).

Rifampicin is one of the broad-spectrum antibiotics and it is a key component of antituberculosis treatment. It inhibits the bacterial RNA polymerase (Campbell *et al*., 2001). Rifampicin causes many adverse effects these are categorized as major adverse effects and minor adverse effects. The major drug effects are skin rashes with or without itching, jaundice, hepatitis, shock, and acute renal failure. The minor adverse effects include abdominal pain, anorexia, vomiting, nausea, flu like

*Corresponding author: Email: khalidrehan71@gmail.com

syndrome. Rifampicin causes acute renal failure and other renal or nephrotoxicity problems (Muthukumar T *et al.,* 2002).

A lot of research work has been done on pharmacognosy, chemistry, pharmacognosy and clinical therapeutics regarding native medicinal plants. Several herbal remedies have been tried in the world system of medicine for the cure of kidney failure. Several approaches have been adopted for the improvement of the herbal system of medicines (Pushpangadan and Kumar, 2005). Herbal plants play important role in the treatment of kidney disorders due to their nephroprotective constituents. Not a single synthetic drug is available which cures full insufficiency of the kidney. Therefore, there is a need to evaluate herbal drugs for their nephroprotective potential because it is observed that where synthetic medicine fails, medicinal plants cure kidney problems. Scientists aimed the use of medicinal plants for curing nephrotoxicity in a threatening stage.

A herbal drug, silymarin is being used for its effects for many years. Silymarin is extracted from the seeds of *Silybum marianum* commonly called as milk thistle and is commercially available in many dosage forms prepared by different pharmaceutical companies. Route of absorption of silymarin is oral route and mainly excreted through bile (Fraschini *et al.,* 2002). The silymarin acts as antioxidant or silymarin acts as permeability stabilizers and hence prevent the entry of toxic agent in the cell. It has been observed that silymarin was found to antagonize the nephrotoxic effects of rifampicin (Karimi *et al*., 2011).

The *Raphanus sativus* leaves and stem also possess strong antioxidant activity which make it a potent nephroprotective agent for nephrotoxicity induced by rifampicin. The present study was designed to assess the nephroprotective potential of *Raphanus sativus* (radish) leaves against nephrotoxicity induced by rifampicin.

Materials and Methods

Plant Extracts: Radish leaves (*Raphanus Sativus*) were identified by the Department of Botany, University of Agriculture, Faisalabad, Pakistan for their authenticity which were bought from the local market of Faisalabad. The distilled water was used to wash the leaves and leaves were shade dried for fifteen days. The fine powder was obtained by grinding the dried leaves in an electric grinder. The powder was weighed and sieved through mash # 20 for the preparation of plant extracts. Water and ethanolic extracts were prepared from the powder. For the preparation of water extract powder was dissolved in distilled water in a ratio of 1:5 respectively. Continuous stirring by vortex shaker at 150 rpm was done for 72 hours and the liquid obtained was filtered through Whatman's filter # 42. Lyophilization of dried material obtained was done in freeze-drying apparatus (Christ Germany model # Alpha 1-4LSC). The lyophilized material was stored in an airtight container at 4ºC.

Ethanolic extract was prepared using the Soxhlet's apparatus. Active materials after weighing and sieving were placed in a thimble and the thimble was held in the extraction chamber. The thimble was held above the flask comprising ethanol of analytical grade and below the condenser, the extracted material was collected in an ethanol filled. The thimble was separated after completion of three cycles and ethanol was evaporated or (After completion of three cycles and ethanol was evaporated, the thimble was separated). The residual substances were lyophilized by freeze drying apparatus. The lyophilized material was stored in an airtight container at 4ºC.

Experimental Animals: Forty male adult albino rabbits procured from the local market of Faisalabad were acclimatized before experiment. Rabbits were kept in the animal room at room temperature (20±2ºC) with ventilation facility at the Institute of Physiology and Pharmacology, the University of Agriculture Faisalabad. Seasonal fodder and water were given ad-libitum. The experimental procedures on rabbits were performed following the institutional animal ethical committee.

Drug administration: Forty male albino rabbits were split up into five equal groups having eight rabbits in each group n=8). The groups were named G1, G2, G3, G4, and G5. G1 was served as a control group and was kept on the normal diet without any treatment while other groups were given respective treatments. Group G2 was given rifampicin alone while group G3 was given rifampicin and silymarin. Group G4 was kept on rifampicin plus ethanolic extract of *Raphanus sativus* while group G5 was given rifampicin plus water extract of *Raphanus sativus*. This feeding and treatment schedule has been shown in Table-1. All the treatments were given orally with the help of a gastric tube. The duration of treatments was 28 days.

Biochemical Analysis: Blood samples collected from the jugular vein of each rabbit in each group were centrifuged to obtain serum samples on the 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of the experiment and serum samples were stored at - 20ºC. Kidney function was measured by finding levels of blood urea nitrogen (BUN), serum creatinine (CR), and serum urea of each rabbit in each group.

Histopathological Analysis: On the 29^{th}-day kidney biopsy of each albino rabbit was taken out to collect the tissue samples and formalin-fixed kidney biopsies were handled in graded ethanolic concentrations and embedded in paraffin blocks. The sections of the kidney were adapted to perpendicular to the plane of section in the block and the transverse section of 6 µm in thickness were cut and mounted on a glass slide then stained with hematoxylin (H) and eosin (E). Olympus PM – 10ADS

Table 1: Feeding and drug administration schedule in male albino rabbits during the experimental period of 0 to 28 days.

Groups	Treatment
Group 1: Control group on routine diet	Routine diet for 0-28 days.
Group 2: Rifampicin given @ (500 mg/kg b.w. P.O.)	Routine diet + Rifampicin (500 mg/kg b.w.) P.O. for 0-28 days as nephrotoxic drug.
Group 3: Rifampicin (500 mg/kg b.w.) + Silymarin (100 mg/kg b.w.) were given P.O.	Routine diet + nephrotoxic drugs + standard nephroprotective drug for 0-28 days.
Group 4: Treated with Rifampicin (500 mg/kg b.w.) + Ethanolic extract of *Raphanus sativus* leaves (2.0 g/kg b.w.) P.O.	Routine diet + nephrotoxic drugs + Ethanolic extract of *Raphanus sativus* leaves for 0-28 days.
Group 5: Rifampicin (500 mg/kg b.w.)+ Aqueous extract of *Raphanus sativus* leaves (2.0 g/kg b.w.) were given P.O.	Routine diet + nephrotoxic drugs + Aqueous extract of *Raphanus sativus* leaves for 0-28 days.

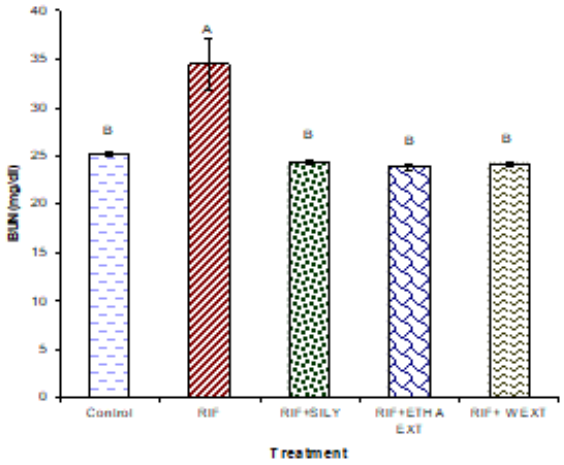

Figure 2: Mean ± SEM comparison of blood urea nitrogen (BUN) mg/dl with oral drugs (rifampicin alone, rifampicin plus silymarin), water and ethanolic extract of *Raphanus sativus* for 28 days in each group (N=8).
Control: No treatment
RIF: (Rifampicin 500 mg/kg b.w. PO)
RIF + SILY: (Rifampicin 500 mg/kg b.w. PO) + (Silymarin 100 mg/kg b.w. PO)
RIF + ETHA EXT (Rifampicin 500 mg/kg b.w. PO) + (ethanolic extract of *Raphanus sativus* 2 g/kg b.w. PO**)**
RIF + W EXT: (Rifampicin 500 mg/kg b.w. PO) + (Water extract of *Raphanus sativus* 2 g/kg b.w. PO)

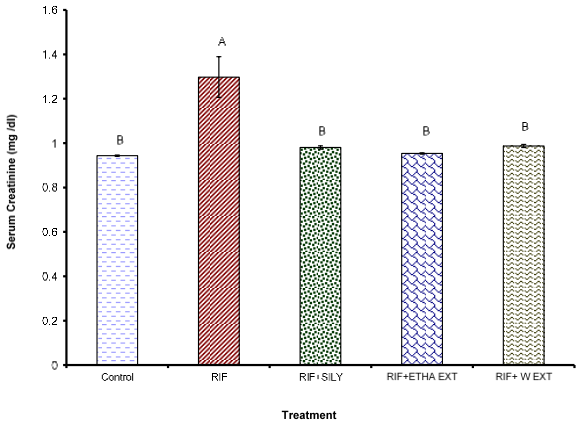

Figure 3: Mean ± SEM comparison of serum creatinine (CR) mg/dl with oral drugs (rifampicin alone, rifampicin plus silymarin), water and ethanolic extract of *Raphanus sativus* for 28 days in each group (n=8).
Control: No treatment
RIF: Rifampicin 500 mg/kg b.w. PO)
RIF + SILY: Rifampicin 500 mg/kg b.w. PO) + (Silymarin 100 mg/kg b.w. PO)
RIF + ETHA EXT: Rifampicin 500 mg/kg b.w. PO) + (ethanolic extract of *Raphanus sativus* 2 g/kg b.w. PO**)**
RIF + W EXT: Rifampicin 500 mg/kg b.w. PO) + (Water extract of *Raphanus sativus* 2 g/kg b.w. PO)

automatic light microscope (Olympus Optical Co., Tokyo, Japan) with a 40X objective was used for microscopy.

Drug concentration: The concentration of Rifampicin in serum was measured by HPLC. The method employed was reverse phase for measurement of drug concentration by HPLC. The assay involved simple liquid extraction of the drug, from specimens and their successive separation on C 18 reversed-phase column and single wavelength UV detection (Calleja et al., 2004).

Statistical Analysis: The values were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was performed for statistical analysis and statistical differences between different treatment groups were determined by Duncan's Multiple Range Test at 5% level of significance.

Results

Biochemical assays

Blood urea nitrogen (BUN): The group was treated with rifampicin 500 mg/kg b.w. alone have BUN ± SEM value (34.46 ± 2.71), which is higher than the rest of the treatments. A significant difference was observed among all groups compared to the rifampicin-treated group, whereas a non-significant ($P<0.05$) difference was observed among all groups in weeks. The results shows that the Mean values of BUN have significantly ($p<0.01$) decreased in groups treated with the rifampicin 500 mg/kg b.w + aqueous extract of *Raphanus sativus* 2 g/kg b.w. and rifampicin 500 mg/kg b.w. + ethanolic extract of *Raphanus sativus* 2 g/kg b.w. as compared to the group treated with rifampicin 500 mg/kg b.w. alone as shown in (Fig-2).

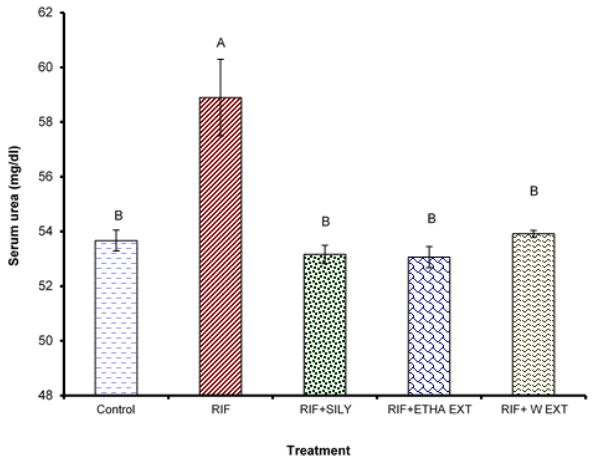

Figure 4: Mean ± SEM comparison of Serum urea (SU) mg/dl with oral drugs (rifampicin alone, rifampicin plus silymarin), water and ethanolic extract of *Raphanus sativus* for 28 days in each group (n=8).
RIF: (Rifampicin 500 mg/kg b.w. PO)
RIF + SILY: (Rifampicin 500 mg/kg b.w. PO) + (Silymarin 100 mg/kg b.w. PO)
RIF + ETHA EXT: (Rifampicin 500 mg/kg b.w. PO) + (ethanolic extract of *Raphanus sativus* 2 g/kg b.w. PO**)**
RIF + W EXT: (Rifampicin 500 mg/kg b.w. PO) + (Water extract of *Raphanus sativus* 2 g/kg b.w. PO

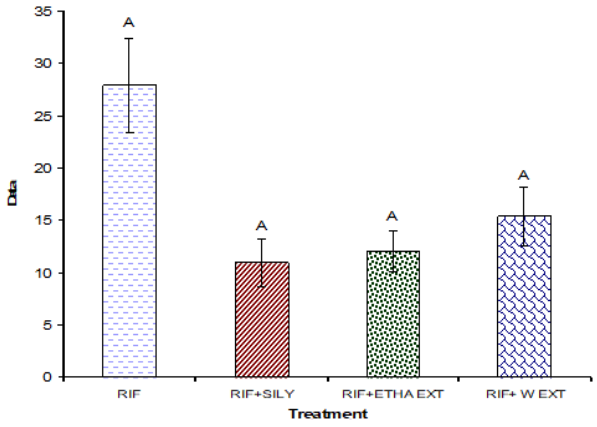

Figure 5: Mean ± SEM comparison of rifampicin concentration in plasma.

Serum creatinine (CR): The group was treated with rifampicin 500 mg/kg b.w. alone have CR ± SEM value (1.298 ± 0.092), which is more than the rest of the treatments. The data also shows that the mean values of serum creatinine have significantly ($p<0.01$) decreased in groups treated with the rifampicin 500 mg/kg b.w. + aqueous extract of *Raphanus sativus* 2 g/kg b.w. and rifampicin 500 mg/kg b.w. + ethanolic extract of *Raphanus sativus* 2 g/kg b.w. as compared to the group treated with rifampicin 500 mg/kg b.w. alone as presented in (Fig-3).

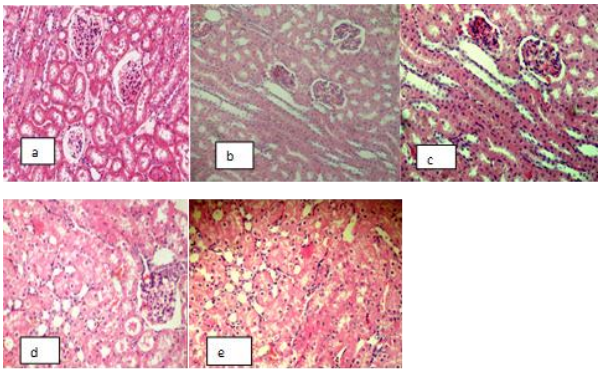

Figure 7: Kidney of rabbit (a) control group, (b) Group 2, (c) Group 3, (d) Group 4, (e) Group 5 (H & E, x 20).

From the data, it was observed that all three treatments were highly significant ($P<0.01$), whereas a non-significant ($P<0.05$) difference was observed among all groups in weeks.
Blood urea (BU): The group was treated with rifampicin 500 mg/kg b.w. alone have CR ± SEM value (58.90 ± 1.40), which is more than the rest of the treatments. The results also shows that the Mean values of serum creatinine have significantly ($p<0.01$) decreased in groups treated with the rifampicin 500 mg/kg b.w. + aqueous extract of *Raphanus sativus* 2 g/kg b.w. and rifampicin 500 mg/kg b.w. + ethanolic extract of *Raphanus sativus* 2 g/kg b.w. as compared to the group treated with rifampicin 500 mg/kg b.w. alone as shown in (Fig-4).

From the data, it was observed that all three treatments were highly significant ($P<0.01$), whereas non-significant ($P<0.05$) difference was observed among all groups in weeks.
Drug estimation: Concentrations of rifampicin are shown in (Fig-5) It was observed that the group which was treated with rifampicin have a high plasma concentration of rifampicin. When silymarin was given the concentration of rifampicin in plasma was decreased. Water extract of *Raphanus sativus* also decreased the plasma concentration of rifampicin but the ethanolic extract of *Raphanus sativus* has more effect on reducing the plasma concentration of rifampicin than water extract. The plasma concentration of rifampicin for ethanolic extract was comparable with that of silymarin.
Histopathological Findings: Photomicrograph of the kidney tissue obtained from the rabbit for all the groups as presented in Figure 7 (a-d).

Discussion

Rifampicin is one of the first-line drugs used for the treatment of tuberculosis. It causes acute interstitial nephritis. When Rifampicin with the @ 500 mg/kg b.w. was given to male albino rabbits for 28 days the kidney

injury markers, blood urea nitrogen (BUN), serum creatinine (CR) and blood urea (SU) were observed. The Mean ± SEM value for (BUN), (CR), (BU) in rifampicin alone treated group were found (34.46 ± 2.71), (1.298 ± 0.09) and (58.90 ± 1.40) respectively while these marker level for control group are (25.18 ± 0.15), (0.944± 0.003) and (53.67 ± 0.38) respectively. Increased levels of these parameters with rifampicin 500 mg/kg b.w. indicate that rifampicin is a nephrotoxic drug as it increases the level of blood urea nitrogen (BUN), serum creatinine (CR) and blood urea (BU). It can be concluded from various studies that rifampicin could cause increased levels of reactive oxygen species which trigger lipid peroxidation of the cell membrane resulting in damage to renal tissue. In case of renal disease, the serum urea amasses i.e., uremia because the rate of urea production is more than the rate of clearance of urea (Mayne, 1994). The urea is a more reliable parameter in determining the dysfunction of the kidney than the serum creatinine (Adelman *et al.,* 1981). Thus, rifampicin at the dose of 500 mg/kg b.w. cause nephrotoxicity in rabbits through oral route for 28 days.

Keeping in view the observed protective effects, silymarin was given at the dose of 100 mg/kg b.w. in the present study to evaluate nephroprotective effects of silymarin. Silymarin was given at the dose of 100 mg/kg b.w. was given along with rifampicin 500 mg/ kg b.w. through the oral route and renal function tests were performed and compared with the control group. It was observed that the mean values of these markers were comparable with the control group. The Mean ± SEM values of (BUN), (CR) and (BU) are (24.33 ± 0.25), (0.981 ± 0.007) and (53.16 ± 0.34) respectively. These values are comparable with the control group. It was concluded that silymarin reduces the toxicity of rifampicin and hence silymarin might be a nephroprotective drug.

The water and ethanolic extract of the leaves of *R. sativus* was given to the rabbits along with rifampicin 500 mg/ kg b.w. for 28 days. The ethanolic and water extract of *Raphanus sativus* has a nephroprotective effect. The Mean ± SEM values for groups with ethanolic extract at the dose of 2 g/kg b.w. the values for blood urea nitrogen (BUN), serum creatinine (CR) and serum urea (SU) was found (23.85 ± 0.35 mg/dl), (0.954 ± 0.003) and (53.06 ± 0.004 mg/dl) respectively, whereas the group treated with water extract at the same dose for 28 days, the Mean ± SEM for blood urea nitrogen (BUN), serum creatinine (CR) and blood urea (BU) was found (24.24 ± 0.19 mg/dl) , (0.987 ± 0.007 mg/dl) and (53.91 ± 0.13 mg/dl) respectively. The group which was treated with ethanolic extract has slightly more nephroprotective than water extract. The ethanolic extract of *Raphanus sativus* reduced the level of these nephrotoxic markers slightly more than water extract and silymarin.

Oxidative stress happens in cells when there is a disturbance of cellular redox balance (Liu *et al.,* 1999) which leads to kidney tissue damage due to oxidation. The proposed mechanism by which these ethnomedicinal plants reduce nephrotoxicity may be due to their antioxidant properties due to flavonoids, alkaloids, saponins, and tannins present in these plants (Borrelli & Izzo, 2000; Shokunbi & Odetola, 2008; Abdulla et al., 2010). Flavonoids like quercetin, is nephroprotective and have been depicted of inhibiting drug-induced nephrotoxicity in experimental animals (Devi and Shyamala, 1999) due to their potent antioxidant effects (Annie *et al.,* 2005). Alkaloids have also been stated to strongly inhibit lipid peroxidation induced in isolated tissues due to its antioxidant activity (Kumaran and Karunnakaran, 2007). Therefore, the possible mechanism of action of *Raphanus sativus* extract is that it has an antioxidant activity that protects the cell from oxidative damage. The leaves of *Raphanus sativus* contain Quercetin which protects the cell lining and hence with the treatments of water extract and ethanolic extract maintained the normal morphology of the kidney.

Histopathological study of the kidney tissues was also performed to examine the modifications in the kidney structure. The tissue sample of the kidney for the control group (Fig-7- a) shows the normal morphology of the kidney, Glomeruli are normal in appearance, proximal convoluted tubules and nuclei are normal on appearance. The photomicrograph (Fig-7- b) represents the groups treated with rifampicin alone shows the congestion in glomeruli and congestion in renal tubules, which are the symptoms of toxicity. When silymarin was given along with rifampicin, the kidney shows normal morphology (Fig-7-c). The biopsy of the kidney treated with ethanolic extract shows no sign of toxicity and is comparable with the control group (Fig- 7-d). The (Fig-7-e) represent the group treated with water extract, by this it is observed that kidney structure is retuning towards normal with the treatment with water extract of *Raphanus sativus*. Leaves of *Raphanus sativus* contain quercetin, Leutolin and Kaempferols, are antioxidant in nature. They protect the living cells from oxidative damage (Matsufuji *et al*., 2003).

The concentration of Rifampicin in serum was measured by high performance liquid chromatography (HPLC). The assay involved simple liquid extraction of a drug, from biological specimens and their following separation on C 18 reversed phase column and single wavelength UV detection. Analytes were determined using UV/Vis detector (SPD-10AV UV). The results of HPLC showed that the group which was treated with rifampicin has a high plasma concentration of rifampicin and when silymarin was given the concentration of rifampicin in plasma was decreased. Both water, as well as ethanolic extract of rifampicin, has also decreased the

plasma concentration of rifampicin. Diuretics are drugs that increase the formation of urine. They act either by increasing the glomerular filtration rate and thus increased the production of urine. *Raphanus sativus* has excellent diuretic properties (Kumar *et al.,* 2010). It increased the urine production; rifampicin concentration was reduced at the fourth week and the toxicity was reduced with the ethanolic extract of *Raphanus sativus*. The ethanolic extract of *Raphanus sativus* reduced the concentration of rifampicin slightly more than water extract.

Conclusion: It was concluded based on all the above studies that rifampicin is a nephrotoxic drug. Ethanolic and water extract of *Raphanus sativus* have a nephroprotective effect against rifampicin. The nephroprotective effect of both extracts was comparable with that of silymarin. There is still a need to evaluate *Raphanus sativus* for the exact components of its leaves present in both extracts have a nephroprotective effect.

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