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Hepatoprotective effects of *Raphanus sativus* (Radish) leaves against toxicity induced by rifampicin in albino rabbits

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Abstract

Tuberculosis (TB) is a contagious and airborne disease. First line therapy for TB includes isoniazid, pyrazinamide, rifampicin and ethambutol administered for 6-9 months depending upon the severity of disease. This standardized therapy is associated with a number of problems including neuropathy, gastrointestinal and hematological disorders. The current study was planned to evaluate protective effects of *Raphanus sativus* (radish) leaves against adverse effects associated with antituberculosis therapy. A total of forty rabbits were divided into five groups. Group 1 was kept as control. Group 2 was given rifampicin orally for 28 days while groups 3, 4 and 5 were given silymarin, ethanolic and aqueous *Raphanus sativus* leaves extract along with rifampicin orally for 28 days respectively. Blood samples were collected before drug administration and at 7th, 14th, 21st, and 28th days post treatment for hematological and biochemical analysis. Liver tissues were taken for histopathological studies. Results have indicated that group treated with rifampicin causes hepatocellular damage which induces the release of aminotransferases. While group treated with ethanolic *Raphanus sativus* leaves extract significantly reduced this elevation in enzymes showing hepatoprotective properties against rifampicin induced toxicity. Hematological studies indicated non-significant differences in erythrocytes, leukocytes, platelets, hemoglobin, erythrocyte sedimentation rate and pack cell volume in treated versus control groups. Histopathological results showed necrosis, hepatic vascular degeneration, congestion throughout cytoplasm and swallowing induced as an adverse effect associated with concomitant use of rifampicin. In conclusion, the alcoholic as well as aqueous extracts of *Raphanus sativus* leaves have shown potent hepatoprotective effects when administered along with rifampicin and have shown results similar to that of silymarin.

Keywords

Hepatoprotective
Raphanus sativus
leaves
Rifampicin
Silymarin
Tuberculosis

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Introduction

Tuberculosis (TB) is a contagious and airborne disease. First line therapy for TB includes isoniazid, rifampicin, pyrazinamide and ethambutol however excellent results for the patients with non-drug resistant tuberculosis can be obtained with a six months course of treatment (Brunton et al., 2006). As the treatment of tuberculosis is of long duration therefore the adverse drug reactions could be troublesome and can even lead to change the treatment plan. The most common adverse effects of the anti-tuberculosis drugs include hepatotoxicity, skin reactions, gastrointestinal and neurological disorders (Arbex et al., 2010; Ramappa & Aithal, 2013; Farazi et al., 2014). The toxic dose of rifampicin causes damage to the structural integrity of liver which is reflected by the increased serum enzyme levels and histopathological studies. Rifampicin is

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metabolised by lever enzymes which causes increase levels of reactive oxygen species in the cell which cause oxidative stress and damages cell leading to the death of the cell. When the hepatocellular plasma membrane is damaged due to any toxicity or necrosis, a variety of liver enzymes such as lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) are released into the blood stream which helps to quantify the extent of damage done to the liver (Amacher, 2002).

Medicinal plants are extensively used for the treatment and prevention of many diseases. Silymarin is one of these noble medicines which is most widely used now a day for its hepatoprotective effects and is obtained from the plant source. *Raphanus sativus* (radish) is a vegetable which is used in cooking and served as salads. Radish leaves are used in salads and are very economical. Radish leaves have been reported for its diuretic and laxative properties, seeds for carminative activities whereas roots have shown therapeutic effects in urinary diseases. It has also hepatoprotective effects and can be used in combination with hepatotoxic drugs to avoid their adverse effects (Rafatullah et al., 2008).

Radish leaves have been evaluated for its hepatoprotective effects. These effects have been observed in rabbits where the hepatotoxic effects were produced by paracetamol that inhibits the hepatic cells defense system leading to the destruction of cell which was evident by the elevated enzymatic levels in the serum (Wlodek & Rommelspacher, 1997). The toxicity induced by the use of paracetamol was treated with the ethanolic and water extract of *R. sativus* leaves and it was observed that ethanolic extract produced more hepatoprotective effects from the water extract which was evident through the reduced levels of transaminases and histopathological evaluation of liver. Keeping in view the above facts, this study has been planned to assess the hepatoprotective effects of radish (*R. sativus*) against the hepatotoxic effects of rifampicin therapy in animal (rabbit) model.

Materials and Methods

Plant extracts: *R. sativus* (radish) was procured from the local market and identified for its authenticity from the Department of Botany, University of Agriculture, and Faisalabad, Pakistan. Leaves of radish were separated and washed with the distilled water and were shade dried for fifteen days. Dried leaves were grinded with electric grinder and powder thus obtained was sieved through mash # 20 which was weighed and allowed for the aqueous and ethanolic extraction processes. For the aqueous extraction, powder obtained after sieving was soaked in distilled water with 1:5 of powder and distil water respectively for 72 hours with continuous stirring by vortex shaker at rate of 150 revolutions per minute and the liquid thus obtained is filtered through whattmans filter # 42. Filtrate obtained was lyophilized by freeze drying apparatus (Christ Germany model# Alpha 1- 4LSC). The dried material obtained after freeze drying was stored in an air tight container at 4°C. For the ethanolic extract, soxhlet apparatus was used. Active powder material after sieving was placed in the thimble; thimble was placed in extraction chamber, which was suspended above the flask containing ethanol of analytical grade and below the condenser, the extracted material is collected in the flask with ethanol, thimble was removed after three complete cycles and ethanol was evaporated. The remaining contents were lyophilized by freeze drying apparatus (Christ, Germany model# Alpha 1–4LSC). The dried material obtained after lyophilization was stored in an air tight container at 4°C.

Experimental animals: Adult male albino rabbits were purchased and kept in the animal room at room temperature (22±2°C) with proper ventilation facility. Rabbits were fed with seasonal fodder and water *ad libitum*. The whole experiment was conducted in accordance with the institutional animal ethical committee.

Drug administration: Feeding and drug administration schedule has been shown in Table 1. Rifampicin was administered at dose rate of 500 mg/kg body weight (BW) each per oral (PO) to the rabbits through stomach tube for 28 days.

Hematological analysis: Blood samples were collected in heparinized tubes before drug administration and then after 7, 14, 21 and 28 days of drug administration to determine hematological parameters including, platelet count, Erythrocyte count (RBC), leukocyte count (WBC), Packed cell volume (PCV), hemoglobin concentration (Hb) and Erythrocyte sedimentation rate (ESR) though hematology auto analyzer.

Biochemical analysis: Serum was separated by centrifugation method and stored at -20°C. Liver function test was assessed by SGPT and SGOT before and after the treatment of rabbits with the drugs which was measured for hepatocellular membrane integrity. All parameters of biochemical analysis was carried out with automatic chemistry analyzer Vistalab selectra E (Vital scientific, N.V., Netherland) using biochemical kits (Human, Germany)

Histopathological analysis: After 28th day, liver biopsy of each rabbit was carried out to collect its tissue samples and formalin fixed liver biopsies were processed in graded ethanolic concentrations and embedded in paraffin blocks. The sections of liver were oriented perpendicular to the plane of section in the block and transverse section of 5 micrometer in thickness were cut and mounted on glass slides and then stained with hematoxylin and eosin commonly called H & E stain.

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

Groups	Treatments
Group 1: Normal control on normal routine diet	Routine diet for 0-28 days.
Group 2: Treated with Rifampicin (500 mg/kg body weight B.W.) per oral (P.O.)	Routine diet + Rifampicin (500mg/kg B.W.) P.O. for 0-28 days as hepatotoxic drugs.
Group 3: Treated with Rifampicin (500 mg/kg body weight B.W.) + Silymarin (100 mg/kg B.W.) P.O.	Routine diet + hepatotoxic drugs + standard hepatoprotective drug for 0-28 days.
Group 4: Treated with Rifampicin (500 mg/kg body weight B.W.) + Ethanolic extract of *R. sativus* leaves (2.0 g/kg B.W.) P.O.	Routine diet + hepatotoxic drugs + Ethanolic extract of *R. sativus* leaves for 0-28 days.
Group 5: Treated with Rifampicin (500 mg/kg body weight B.W.) + Aqueous extract of *R. sativus* leaves (2.0 g/kg B.W.) P.O.	Routine diet + hepatotoxic drugs + Aqueous extract of *R. sativus* leaves for 0-28 days.

Statistical analysis: The values will be expressed as mean ± SEM. Statistical analysis will be performed by one way analysis of variance (ANOVA) and statistical differences among different treatment groups will be determined by Duncan's Multiple Range test at 5% level of significance (Steel & Torrie, 1960).

Results

Hematological analysis: Hematological studies indicated non-significant differences in platelets count, RBC count, PCV, Hb and ESR in treated versus control groups as presented in Table 2. While WBC count has significantly ($p<0.05$) decreased in the group treated with the rifampicin alone as compared to the control group.

Biochemical analysis: The Mean values of SGPT and SGOT have significantly ($p<0.01$) increased in the group treated with rifampicin 500 mg/kg BW alone as compared to the control group and the group treated with rifampicin 500 mg/kg + silymarin 100 mg/kg. The data also indicates that the Mean values of SGPT and SGOT have significantly ($p<0.01$) decreased in groups treated with the rifampicn 500 mg/kg BW + aqueous extract of *R. sativus* 2g/kg BW and rifampicin 500 mg/kg BW + ethanolic extract of *R. sativus* 2g/kg BW as compared to the group treated with rifampicin 500 mg/kg BW alone as presented in Table 3.

Histopathological Findings: Photomicrograph of the liver tissue obtained from the rabbit for all the groups have been shown in Figure 1 (a-e).

Discussion

TB is a contagious and chronic air born disease and is one of the major cause of death around the world. Fortunately, the treatment of TB has been well developed now but there are some complications associated with the treatment and one of them is the prolong drug treatment for at least six months as recommended by the World Health Organization. This prolong use of drugs have the potential to cause severe adverse effects and hepatotoxicity is among the most life threatening adverse effects associated specially with the drugs of first line treatment (Stout et al., 2003). Rifampicin is one of the drugs included in the first line treatment of TB. This study has been designed to evaluate the hepatotoxic potential of rifampicin along with its effects on hematology in albino rabbits. SGPT and SGOT enzymes are present in the hepatocyte and they are released into plasma when hepatocytes are damaged and studies have suggested that rifampicin could be responsible for the production of increased levels of reactive oxygen species which cause lipid peroxidation of the cell membrane resulting in the rupture of cell and releasing these enzyme into the plasma. Silymarin was used in this study as standard which have shown its hepatoprotective effects by increasing the levels of antioxidants thus scavenging the high levels of reactive oxygen species and maintaining the cell integrity (Gupta et al., 2002; Rolo et al., 2003; Gupta et al., 2004). Leaves of *R. sativus* have high levels of flavonoids and the results suggest that these flavonoids have a role in the production of antioxidants and therefore have maintained the cellular integrity of hepatocytes as compared with the group treated with the silymarin.

The dose of rifampicin used for the study was 500 mg/kg BW once daily for 28 days. The dose of rifampicin used in the similar study was 1000 mg/kg BW once daily for 15 days and have shown elevated levels of liver enzymes (Anusuya et al., 2010). In another study rifampicin was used at the rate of 500 mg/kg orally for 30 days (Naik & Panda, 2008) and elevated levels of SGPT and SGOT are in accordance with the results of the this study. These two liver enzymes are present normally in hepatocytes and there leakage into the plasma show that the cell membranes of hepatocytes have been ruptured. Silymarin is now a days have been used as a hepatoprotective agent clinically. Silymarin have shown hepatoprotective effects by reducing the levels of reactive oxygen species inside the cell thus giving cell membrane a protection against the lipid peroxidation. Silymarin was used at the dose of 100 mg/kg against the acetaminophen induced hepatotoxicity in wistar rats and the results in this study clearly indicated a significant decrease in the elevated levels of SGPT and SGOT when used with acetaminophen 2g/kg as indicated in present study.

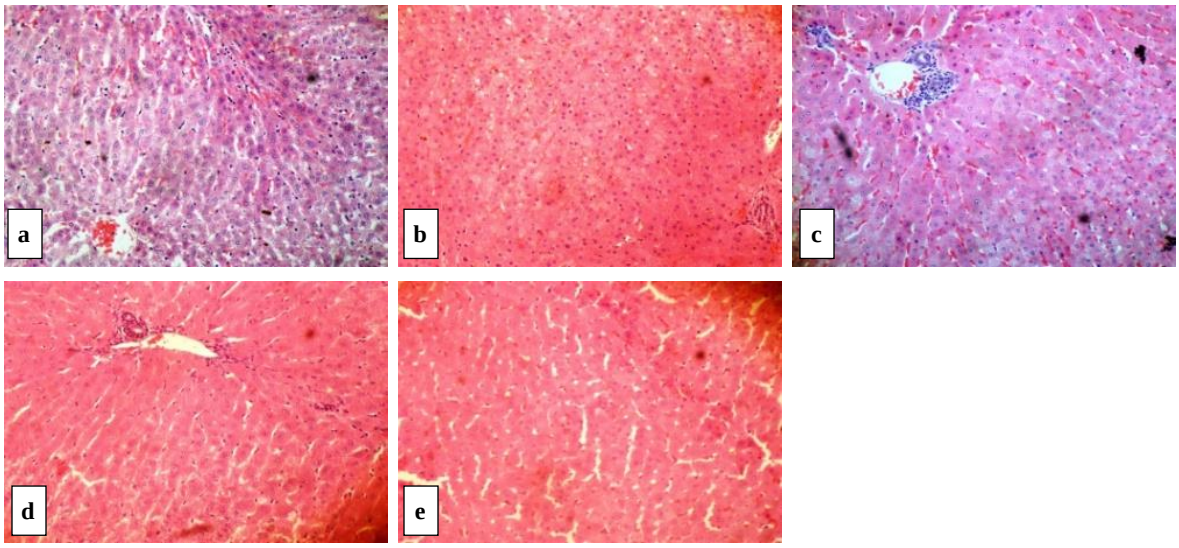

Figure 1: Liver of rabbit (a) control group, (b) rifampicin (500 mg/kg BW), (c) rifampicin (500 mg/kg BW) + silymarin (100 mg/kg BW), (d) rifampicin (500mg/kg BW) + ethanolic extract (2g/kg BW), (e) Rifampicin (500mg/kg BW) + aqueous extract of *R. sativus* (2g/kg BW) with daily oral administration for 28 days (H & E, x 20).

Table 2: Hematological parameters (Mean±SEM) with oral drugs and *R. sativus* extracts daily administration for 28 days in rabbits (n=8).

Parameters	Groups				
	1	2	3	4	5
Platelet count (10^9/L)	283.22 ± 14.95^{A}	185.96 ± 33.62^{B}	262.16 ± 0.90^{A}	275.19 ± 22.75^{A}	310.58 ± 18.33^{A}
RBC count (10^{12}/L)	5.27 ± 0.19^{B}	6.08 ± 0.01^{A}	6.09 ± 0.00^{A}	5.82 ± 0.43^{AB}	6.19 ± 0.07^{A}
WBCs count (10^9/L)	9.44 ± 0.38^{AB}	6.77 ± 1.05^{C}	7.66 ± 0.26^{BC}	8.92 ± 0.77^{AB}	9.61 ± 0.08^{A}
PCV (%)	36.23 ± 0.85^{A}	36.68 ± 0.69^{A}	38.84 ± 0.10^{A}	35.33 ± 2.44^{A}	39.73 ± 1.38^{A}
Hb (g/dl)	12.04 ± 0.13^{A}	10.71 ± 0.03^{A}	10.36 ± 0.54^{A}	10.41 ± 0.84^{A}	11.44 ± 0.05^{A}
ESR (mm/hr)	2.17 ± 0.31^{A}	2.33 ± 0.22^{A}	2.45 ± 0.32^{A}	1.69 ± 0.05^{A}	1.70 ± 0.08^{A}

Means sharing similar letter in a column are statistically non-significant ($p>0.05$). 1=Control, 2= rifampicin (500 mg/kg BW), 3 = rifampicin (500 mg/kg BW) + silymarin (100 mg/kg BW), 4 = rifampicin (500mg/kg BW) + ethanolic extract (2g/kg BW), 5 = Rifampicin (500mg/kg BW) + aqueous extract of *R. sativus* (2g/kg BW).

Many medicinal plants are being tested for their hepatoprotective activity. Ethanolic and aqueous extract with the ratio of (7:3) of *Cytisus scoparius* was used in a study to evaluate its hepatoprotective effect (Raja et al., 2007). In another study, *Raphanus sativus* commonly called radish has also been used to evaluate its hepatoprotective effects. In this study, water and ethanolic extract of *R. sativus* was used at the dose level of 2g/kg BW in each albino rabbit. Hepatotoxicity in this study was induced with the help of acetaminophen at the dose level of 100 mg/kg BW for 45 days. In this study, both the water and ethanolic extract have shown similar hepatoprotective effects but the ethanolic extract has shown more hepatoprotective activity (Anwar & Ahmad, 2006). As *R. sativus* is very common and are very economical so in the present study, *R. sativus* leaves, ethanolic and water extract were used to evaluate its hepatoprotective effects against the hepatotoxicity induced by the rifampicin. In the present study, the effect of rifampicin and extracts of *R. sativus* on hematology was also determined and the results were compared with the standard drug silymarin. WBC count has significantly ($p<0.05$) decreased in the group treated with the rifampicin alone as compared to the control group which might be due to bone marrow depression that is a sign of toxicity of rifampicin. It was also demonstrated in another study where the WBC count have decreased significantly as compared to the control group and it was noted in this study that other antituberculosis drugs like pyrazinamide and isoniazid have decreased the WBC count to lesser extent than that of the rifampicin (Tasduq et al., 2007).

Histopathological studies were also conducted to observe the changes occurred in the liver tissue. The tissue sample obtained from the rabbit of the control group, shows that the hepatocytes are present within hepatic chords having nucleus with nucleolus but the blood vessels are mildly hyperemic. The photomicrograph of the group treated with rifampicin alone clearly shows the sign of toxicity as the congestion is present throughout the parenchyma of the hepatocytes and hepatic cells are swallow but when

Table 3: Mean ± SEM values of SGPT (IU/L) and SGOT with per oral drugs and *R. sativus* extracts daily administration for 28 days in rabbits (n=8).

Groups	Biochemical Examination	
	SGPT (IU/L)	SGOT (IU/L)
1	23.20 ± 0.32^{B}	53.10 ± 0.87^{B}
2	39.83 ± 3.54^{A}	69.70 ± 4.70^{A}
3	26.55 ± 1.88^{B}	53.40 ± 1.14^{B}
4	26.675 ± 1.17^{B}	49.05 ± 1.48^{B}
5	19.70 ± 2.33^{B}	52.38 ± 1.98^{B}

Means sharing similar letter in a column are statistically non-significant (P>0.05). 1=Control, 2= rifampicin (500 mg/kg BW), 3 = rifampicin (500 mg/kg BW) + silymarin (100 mg/kg BW), 4 = rifampicin (500mg/kg BW) + ethanolic extract (2g/kg BW), 5 = Rifampicin (500mg/kg BW) + aqueous extract of *R. sativus* (2g/kg BW).

rifampicin is given with silymarin the hepatic cells were found to be normal and the blood vessels are mildly hyperemic and is comparable with the group treated with aqueous extract of *R. sativus.* The microphotograph of ethanolic extract group has shown normal hepatocytes with normal sinusoidal spaces showing no signs of hepatotoxicity. The proposed mechanism by which it reduces hepatotoxicity may be due to its antioxidant properties because of flavonoids, saponins and tannins (Borrelli & Izzo, 2000; Shokunbi & Odetola, 2008; Abdulla et al., 2010).

Conclusion: The results of the present study were helpful to conclude that rifampicin, an important component of the tuberculosis drug treatment, can cause hepatotoxicity and should be prescribed to the patients with chronic liver disease with caution. The study have also shown that if rifampicin is given with some hepatoprotective drug like silymarin the hepatotoxic effect of it can compromised. The ethanolic and aqueous extracts of leaves of *R. sativus* have successfully shown a potent hepatoprotective effects when given with rifampicin as compared with the silymarin.

Conflict of interest: All authors declare no conflict of interest.

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