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SILYMARIN'S ANTIOXIDANT AND PROTECTIVE EFFECTS ON ISONIAZID AND RIFAMPICIN-INDUCED LIVER AND KIDNEY TISSUE DAMAGE IN ADULT RATS

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ABSTRACT

The objective of the present study was to investigate the possibility that silymarin could mitigate the harm that isoniazid (INH) and rifampicin (RIF) cause to male albino rats' hepatorenal tissues.

Forty male albino rats were divided into four groups weighing (180-200 g) and each received a different treatment: The control group was group 1, which received normal saline orally (NaCl, 0.9%); Group 2 got isoniazid (100 mg/kg, i.p.) along with rifampicin (100 mg/kg, i.p.) in sterile water, Group 3 received silymarin (200 mg/kg/p.o.); and Group 4 also received silymarin (200 mg/kg/p.o.) and was given isoniazid (50 mg/kg/i.p.) and rifampicin (100 mg/kg/i.p.). It took 28 days to do this treatment once each day. Alanine aminotransferase (ALT), aspartate aminotransferase (ALT), total protein (TP), bilirubin, and glutathione (GSH) levels were measured as biomarkers. Additionally, histopathological examinations of the liver and kidney tissues of the experimental animals were carried out.

Results indicated Rats used in experiments received isoniazid and rifampicin, which resulted in a considerable increase in liver marker enzyme levels and a significant decrease in TP and glutathione (GSH) levels. Silymarin use, however, improved these results.

According to a histological investigation, taking Rifampicin and Isoniazid together damaged the hepatorenal tissue and congested the blood vessels. However, taking silymarin along with rifampicin-isoniazid reduced this damage, and improvements were seen in the tissue's levels of oxidative stress, inflammation, and some inflammatory cell markers after treatment with both test drugs.

Keywords: Silymarin's antioxidant, kidney, liver, histopathological, Rifampicin, isoniazid

Introduction

Over a third of the world's population is affected by the highly contagious disease tuberculosis, which claims over 2 million lives annually (Shishoo et al.,2001). The first-line medications used to treat tuberculosis, isoniazid (INH) and rifampicin (RIF), when administered together, were linked to 2-6% hepatotoxicity, according to a meta-analytical analysis (Tasduq et al., 2005). These medications increase the production of highly reactive oxygen species (ROS), which catalyze lipid peroxidation and are a means of destroying the plasma membrane (Georgieva et al., 2004). RIF strongly stimulates CYP2E1, a member of the cytochrome- P450 family that is in charge of metabolizing environmental toxins and carcinogens. Encouraging the production of the harmful metabolite hydrazine via the amidase pathway also amplifies the toxicity caused by INH. Then, as a result of hydrazine's reaction with glutathione's (GSH) sulfhydryl group, the levels of GSH in hepatocytes are reduced, leading to cell death (Tasduq et al., 2005). It was discovered that INH-RIH treatment increased the rate at which INH turned into the hepatotoxic compound isonicotinic acid. Because acetyl hydrazine is swiftly transformed into its active metabolites by RIF, the plasma half-life is further decreased, which increases the likelihood of liver necrosis(Tostmann et al.,2008). The antibiotics rifampicin and isoniazid have also been linked to oxidative kidney tissue damage, according to a study. Additionally, antioxidants that lessen lipid peroxidation (LPO) byproducts such malondialdehyde have been found to lessen kidney tissue damage (MDA)(Martin et al.,2016). Research suggests that antioxidants may protect against the kidney and liver damage that can result from taking isoniazid and rifampicin together.

Traditional medical practices using herbal remedies continue to provide substantial contributions to health in terms of the prevention and treatment of many diseases (Guilford et al.,2008). Silymarin, a well-known milk thistle (Silybum marianum) extract, has been used extensively as a herbal treatment for liver protection(Tsai et al.,2008) Silymarin has been demonstrated to have positive effects by reestablishing oxidative homeostasis and reducing inflammation by inhibiting nuclear factor kappa B activation and regulating the expression of cyclooxygenase (COX) 2, inducible nitric oxide synthase (iNOS), vascular endothelial growth factor, and matrix metalloproteinases(Al-Rasheedet al.,2016; Zima et al.,1998)). The objective of the present investigation was to evaluate the protective effects of silymarin against RIF-INH-induced hepatorenal damage by employing histoarchitecture, markers of oxidative stress, and serum liver markers.

Material and Methods

Experimental animals

40 male albino rats of average weight (180–200 g) were purchased from the Animal House at the University of Mosul's College of Veterinary Medicine. Separated from one another, the animals were kept in polypropylene cages for acclimatization at (20-23 °C), 50–60% relative humidity, and a 12-hour light/dark cycle for a week before to and at the start of the experiment. Over the course of the housing period, animals were given a regular pellet meal and free access to water.

Experimental design and treatment protocol

Four groups made up of six rats each were created at random from the total of all the rats (n=6).

Group 1: was solely given standard saline, 0.9% (1mL/kg body weight) (p.o).For 28 days.

Group 2: only given INH-RIF 100 mg/kg b. wt. (i.p.) once daily For 28 days (Yue *et al*., 2004, Saleem *et al*., 2008)..

Group 3: only was given Silymarin 200 mg/kg b.wt (p.o.) once daily For 28 days (Eminzade,et al.,2008) .

Group 4: was given INH-RIF 100 mg/kg bwt (i. p.) and Silymarin 200 mg/kg b.wt (p.o.) both once daily For 28 days.

Measurement of serum ALT, AST,and TP

serum samples from blood samples taken from a heart puncture (after the treatments) were centrifuged and kept in a deep freezer. Using kits produced by Roche Diagnostic Division, the activity of the blood enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), Total protein(TP), and total bilirubin were measured by the Hitachi-917 auto-analyzer(Shediwa et al.,2019)

Preparing tissue extract

Once the liver tissues had been homogenized in ice-cold 0.1M phosphate buffer, they were centrifuged at 15,000 rpm for 30 minutes at 4 °C (pH 7.4). The supernatant was collected and stored at 80 °C for the antioxidant experiment. was used to calculate the amount of GSH present in the serum and tissue homogenates [Naji et al.,2017].

Histopathological examination of liver and kidney

For histological evaluation, the isolated liver and kidney from rats in various groups were kept in 10% formalin solution. By embedding the liver and kidney in paraffin and cutting it into 5 µm , the organs were mounted in the lab. These were light-microscopically viewed after being stained with the colors eosin and hematoxylin.

Statistical analysis

The mean and standard error were used to express all data (SE). The statistical analysis was performed using SPSS 26 statistical software. Data were analyzed using one-way ANOVA to compare the means among different groups and Tukey's test. A p-value <0.05 was considered significant.

Results

Antioxidant markers

GSH levels shown to have significantly decreased in the RIF +INH group compared to the control group (P< 0.001). Rats given RIF +INH + silymarin displayed a significant increase in compared to the RIF + INH group (p< 0.001). (Fig1).

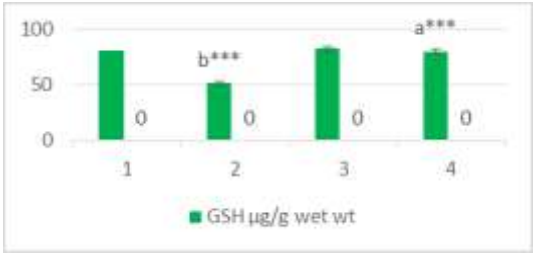

Fig. 1: The mean± S.E of GSH levels in different group of rats:Constant dose of INH(100mg/kg) , RIF(100mg/kg) and silymarin (200mg/kg) were used.

1-Control group, 2-RIF+INHgroup, 3-SILgroup, 4-RIF+INH+SILgroup. * Indicates significant difference with ***P < 0.001;(N=6). a : sgnificant vc RIF +INH group
b : significant vc control group.

Liver Function Tests

The levels of AST, ALT, and total bilirubin were all elevated after receiving 100 mg/kg doses of RIF and INH, respectively. In group 4, silymarin treatment at 200 mg/kg an hour before intraperitoneal injection of RIF-INH resulted in lower levels of AST, ALT, and total bilirubin than in the RIF-INH group (Table). By administering RIF-INH to gruop2 intraperitoneally at a dose of 100 mg/kg, TP was decreased. One hour before intraperitoneal injection of RIF-INH, ,. silymarin treatment at dose of 200 mg/kg p.o. raised the level of Total protein(table).

Table: Effects of silymarin on the levels of various blood marker enzymes after antituberculer drug-induced liver injury in rats.

Group	Total protein(gr/dl)	Total bilirubin(mg/dl)	AST(IU/dl)	ALT(IU/dl)
Control group(G1)	6.76 ±0.43	0.75 ± 0.02	110.40 ± 1.29	43.28 ± 1.98
RIF+INH group (G2)	3.96 ±0.41[a]**	1.09 ± 0.83[a]***	139.55± 0.90 [a]***	57.76 ± 1.22[a]***
Silymarin group (G3)	6.93 ± 0.31	0.90 ± 0.03	112.52 ± 0.55	39.47 ± 1.05
RIF+INH+Silymarin group (G4)	5.57 ±0.47[ab]	0.79 ± 0.02[b]**	136.07 ± 0.51 [b]*	49.17 ± 1.49[b]**

Results are presented as mean ± SE (n=6) for all data.
*<0.05 , **<0.01 ,***<0.001
a : significant vs the Control group, b : significant vs the Rif-INHgroup.

Histological assessment of liver and kidney tissue

When first-line anti-TB medications were delivered to the RIF-INH group (G2), the liver underwent histopathological analysis. This analysis revealed structural changes, including dilated and severe congestion of the central vein, hyperplasia of the kupffer cells, necrosis around the central vein, Pyknosis of the hepatocytes, hemorrhage in the sinusoid of the liver, and Focal infiltration of inflammatory cells (figure2, B, C). Treatment with Silymarin extract (200 mg/kg/body weight) in the RIF-INH + Silymarin group(G4) demonstrated much less damage as compared to the RIF-INH group, including less congestion and their tobular architecture was regular with inflammatory cells(figure2, E, F). Control (G1) and Silymarin group(G3) sections did not exhibit any distinctive changes in the liver's histology(figure2, A, D) both displayed normal glomerular and tubular histology, and the morphology of the kidney tissue was unaltered(figure3, A, D). INH and RIF group (Group II) demonstrated multiple foci of hemorrhage in the interstitial area, infiltration of inflammatory cells around affected tubules, hypertrophy of glomerular tuft with adhesion between glomerular tuft and bowman capsule and congestion, RBCs casts in tubules, and necrosis in tubule epithelial cells, indicating their nephrotoxic effect(figure3, B, C). Group 4 of the RIF+INH and Silymarin (200 mg/kg b.w.) study revealed normal renal parenchyma, little blood vessel congestedness, minor tubule epithelial cell hypertrophy, a small amount of hemorrhage around damaged tubules(h), and some inflammatory cells(figure3, E, F).

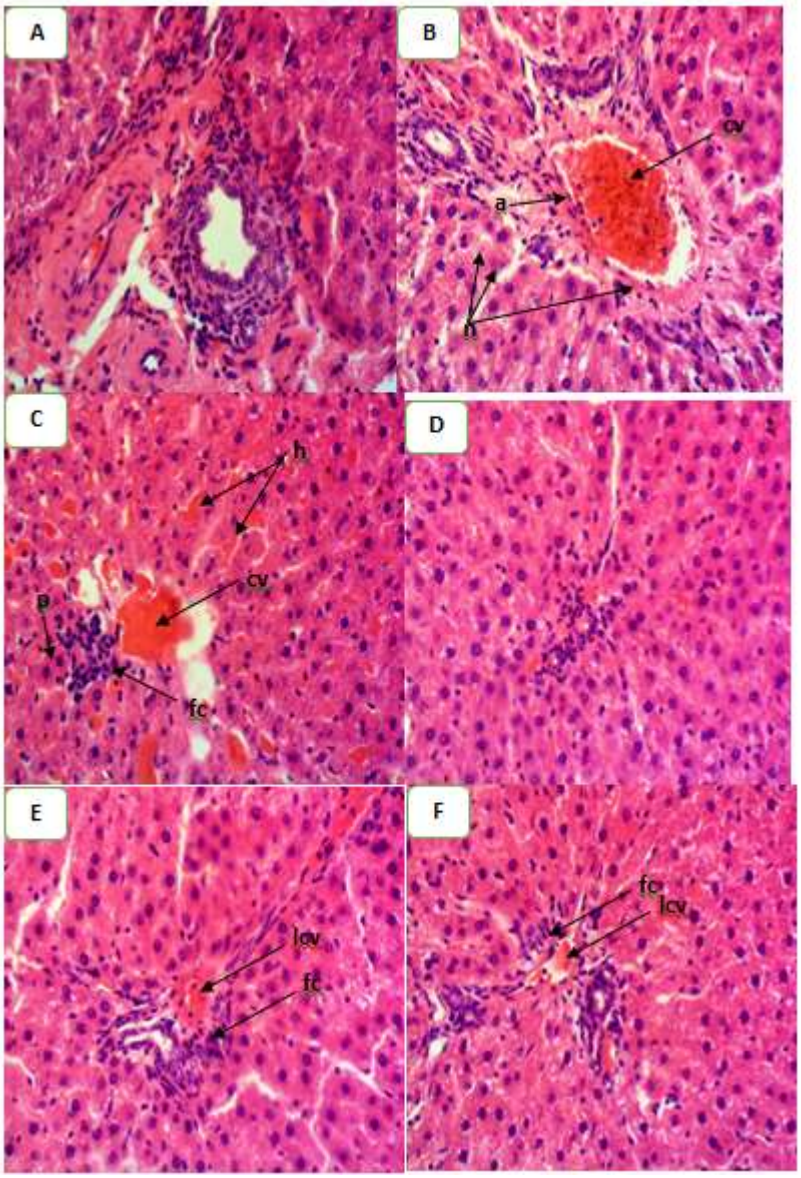

Fig. 2: Liver section showing normal apearance in (A) Control group and(D) Silymarin group.(B) INH+RIF group showing dilated and severe congestion of central vein(cv), kupffer cells hyperplasia (a) and necrosis around central vein(n).(C)INH+RIF group showing heamorrhage in sinusoid of liver(h) ,Pyknosis of the hepatocytes(p), Focal of inflammatory cells (fc) and Congestion of blood vessel(cv).(E),(F) Silymarin +INH and RIF group showing little congestion(lcv) and their lubular architecture was regular with inflammatory cells(fc).(H&E).

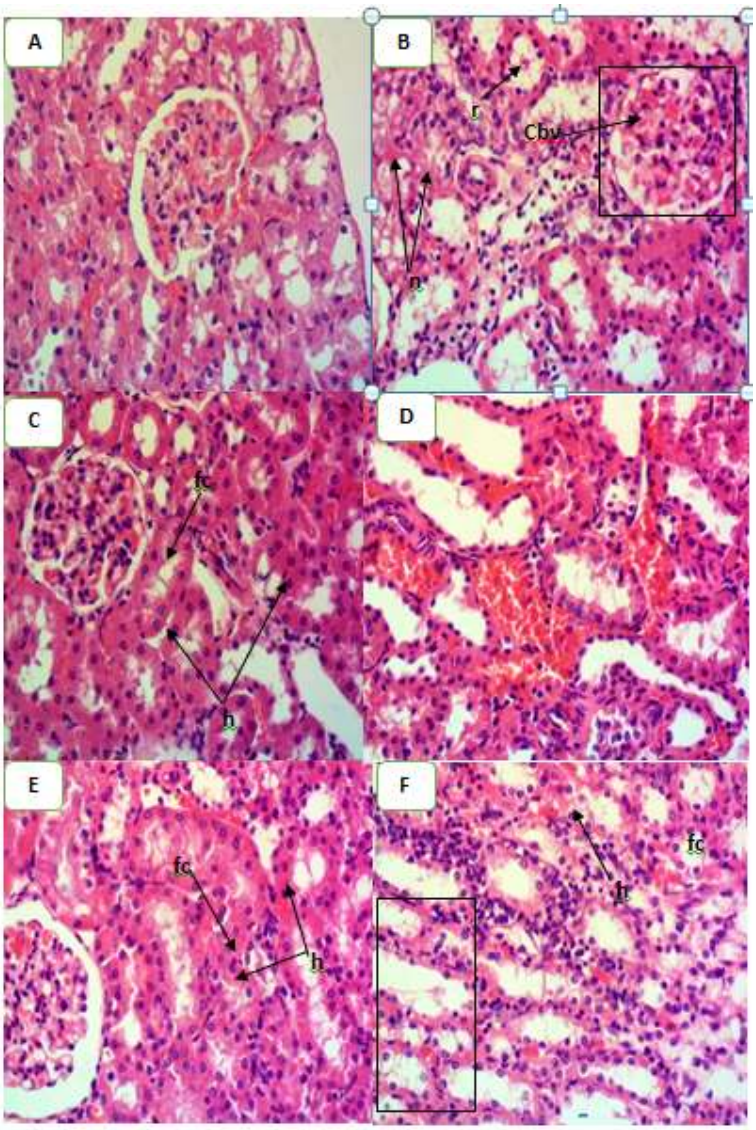

Fig. 3: Kidney section showing normal appearance in (A) Control group and(D) Silymarin group.(C) INH+RIF group showing mltiple foci of haemorrhage in the interstitial area(h) and infiltration of inflammtory cells around affected tubules(fc). (B) INH+RIF group showing hypertrophy of glomerular tuft with adhesion between bowman capsule and glomerular tuft(square), RBCs casts in tubules(r) and necrosis in tubule epithelial cells(n). (E)INH+RIF+ silymarin renal parenchyma showing minimal haemorrhage(h)with inflammatory cells(fc).(F) INH+RIF+silymarin showing minimal glomerular congestion with little enlargement of tubule epithelial cells(square) and a little of haemorrhage around affected tubules(h) with some inflammatory cells(fc).(H&E,400x).

Discussion

In the current investigation, silymarin's ability to protect the rat liver and kidney from the toxic effects of anti-tubercular medicines (INH and RIF) was examined. The hepatotoxicity of these two medications is a significant concern during clinical therapy. These similarities may indicate linked processes of hydrazine and RIF-induced liver damage, which is a potential area of research for the future. A noteworthy finding in this regard is that, notwithstanding the rarity of RIF-induced hepatotoxicity, INH-induced hepatotoxicity is greatly increased when RIF is administered in addition to INH (Sahu et al., 2015). This is thought to occur because RIF ingestion activates a number of CYPs via the PXR, and CYP3A4 activation in particular causes faster INH metabolism and the production of hydrazine (Ramappa and Aithal, 2013). INH-induced hepatotoxicity is currently thought to be primarily caused by hydrazine, a potent reducing agent and the host's principal metabolite of INH. This metabolite has been linked to the development of megamitochondria in rat livers and ATP depletion in hepatocytes (Combrink, and du Preez., 2020). Additionally, RIF accelerated INH's conversion to the hepatotoxic compounds hydrazine and isonicotinic acid. RIF shortens the plasma half-life of mono acetyl hydrazine and accelerates the conversion of mono acetyl hydrazine to its active metabolites, which is

associated to the increased risk of liver necrosis brought on by the interaction of INH and RIF(Hussain et al., 2012). These in turn result in an increase in the liver's enzymes (SGOT, SGPT, and total bilirubin) and a decrease in the blood's total protein levels (Wali., et al. 2015). The current study also found that total protein (TP) levels in group 2 were significantly ($p > .01$) decreased compared to in group 1 whereas SGPT, SGOT, and total bilirubin levels in serum significantly ($p > .001$) increased. This finding is in line with the research mentioned by Thuawaini et al., (2019), therapy with RIF and INH caused an increase in serum ALT, AST, ALP, total bilirubin, and a decrease in total protein. Following silymarin administration to the Rif-INH groups, the rats showed a decrease in SGOT, SGPT, total bilirubin, and a rise in total protein levels; Silymarin significantly reduced abnormal bilirubin levels and returned liver enzyme levels to normal, indicating that they play a hepatoprotective role. This result is consistent with a study that found that giving silymarin along with INH+RIF reduced the hepatotoxicity of medicines as determined by liver function tests(Eminzade et at.,2008). The reduced scavenging capacity of hepatocytes and reduced scavenging capabilities of the hepatocytes are linked to an increase in the body's free radical concentration(Grahm et al.,2001). Due to an increase in free radicals and a lack of scavenging ability in hepatocytes, increased levels of oxidative stress are linked to hepatotoxicity (Murie., 2009; Forrester. et al.,2018). According to a prior study, taking the combination of rifampin and isoniazid increased liver enzyme levels and decreased glutathione levels in the liver, while Silymarin restored these abnormal changes(Tasduq.,2005). In our study we observed, a decrease in GSH content in the RIF-INH group (G2) is a sign of increased oxidative stress in the INH + RIF group. In this investigation, it was found that pretreatment with silymarin at a dose of 200 mg/kg orally, followed by 100 mg/kg intraperitoneally of each (RIF + INH), increased levels of GSH in group 4 .

The histological tests showed that group 2 had sinusoidal space congestion in the centrilobular area, hemorrhage in the sinusoid, and inflammation, in contrast to a group 1, which showed no change in the normal architecture of the liver. Silymarin treatment in group 3 prevented all of the histopathological abnormalities brought on by INH-RIF, which is consistent with the findings of Tukappa et al.(2015).

Nephrotoxicity is a side effect of numerous medications, including antituberculosis therapy (Singh et al.,2003). On the other hand, nephrotoxicity caused by these substances occurs less frequently and has not been well-researched. INH is not known to cause nephrotoxicity, but RIF has been shown to do so in occasional cases of acute renal failure (ARF)(De Vriese et al.,1998; Chang et al.,2014). Under a microscope, isoniazid and rifampicin group's kidney tubular epithelial cells displayed interstitial necrosis and hemorrhage**.** In addition, glomerular obstruction and intratubular protein cast formation were brought on by the administration of isoniazid and rifampicin together. However, silymarin significantly reduced the pathological damage caused by the interaction of isoniazid with rifampicin. According to recent studies, the medication combination damages kidney tubular epithelial cells(Sahu et al.,2020). shown that a combination of anti-tuberculosis medications causes significant histological damage to kidney tubules and glomeruli, but necrosis is the most frequent observation (Sharma et al.,2019). It has been demonstrated that rifampicin causes partial tubular epithelial cell desquamation, peritubular congestion, localized tubular epithelial cell degeneration, and congested kidney glomeruli (Ramasamy et al.,2018).

Conclusion

This study's results provide evidence that silymarin helps delay the onset of acute hepatorenal failure in cases of antitubercular drug-induced hepatorenal toxicity and may have therapeutic potential.

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Conflict of interest

The authors affirm that there were no financial or editorial conflicts of interest in the creation or publication of this paper.

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