ADDITIVE HEPATOPROTECTION OF RANITIDINE WITH VITAMIN E IN RIFAMPICIN INDUCED HEPATOTOXICITY IN RATS

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Summary

The aim of the present study was to determine whether ranitidine and vitamin E might be additive in their protection against rifampicin hepatotoxicity. Hepatotoxicity was produced by giving 50 mg/kg body weight/day of rifampicin for 4 weeks. The treatments of Ranitidine alone (50 mg/kg, 75 mg/kg, 100 mg/kg and 120 mg/kg body weight/day), vitamin E alone (50 mg/kg, 75 mg/kg and 100 mg/kg body weight/day) and combinations of ranitidine plus vitamin E were given 30 minutes prior to the administration of rifampicin. The effect on serum marker enzymes, antioxidant activities such as lipid peroxidation, superoxide dismutase (SOD) and reduced glutathione (GSH) as well as cytochrome P-450 content were assessed. Treatment with ranitidine alone or vitamin E alone slightly improved serum marker enzymes, antioxidant activities and cytochrome P-450 content in dose related manner but the protection by the combination of ranitidine and vitamin E was additive when compared to each agent alone. Combined treatment with ranitidine plus vitamin E greatly protected the liver from tissue injury and exhibited a significant effect showing reducing serum marker enzymes, LPO, cytochrome P-450 content and increasing levels of SOD as well as reduced GSH levels. From the results obtained, it can be concluded that protection against rifampicin hepatotoxicity using a combination of ranitidine and vitamin E is better than that found with either agent alone. We presume that the inhibition of cytochrome P-450 is the main factor in the protection by ranitidine and vitamin E against rifampicin inuced hepatic injury.

Keywords: Rifampicin; Ranitidine; Vitamin E; Antioxidant; Cytochrome P-450; Hepatotoxicity

Introduction

Tuberculosis is one of the major health problems in developing countries like India. Most adult deaths in India are due to vascular diseases or pulmonary tuberculosis. It was reported that clinical hepatitis occurs in 1.1% of adults receiving Rifampicin containing regimens. Occasional cases of Rifampicin-associated hepatitis have been reported in patients not receiving isoniazid (INH) treatment. [1]. RMP causes a direct toxic injury to the hepatocytes [2]. This has been confirmed in experimental rats [3]. Oxidative stress has been found to be an important mechanism in hepatotoxicity of anti-tubercular drugs. The altered profile of antioxidant enzymes along with increased lipid peroxidation indicated enhanced oxidative stress in treatment with combination of isoniazid [INH] and RMP [3,4]. Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione [GSH]] have been reported in isoniazid and rifampicin-induced hepatoxicity [5].

The antioxidant effects of several antihistamine drugs were studied [6]. The histamine H_2-receptor antagonists are able to prevent gastric mucosal lipid peroxidation [7]. H_2 receptor antagonists [i.e. cimetidine, ranitidine, and famotidine] have been found to be good hydroxyl [•OH] radical scavengers [8]. Ranitidine is a more powerful inhibitor of cytochrome P-450 enzyme systems. It was also reported that the acetaminophen hepatotoxicity was reduced with dose dependent manner with use of ranitidine administered at doses ranging from 50 to 120 mg/kg body weight and also mortality can be reduced predictably from 90% to 25% [9]. Also, it was reported that high doses of Ranitidine [120 mg/kg] decreased acetaminophen hepatotoxicity in rats [10]. So, ranitidine was chosen for these experiments instead of cimetidine, which is a more powerful inhibitor of P-450 enzyme systems, in an effort to avoid the “hormonal” antiandrogenic influence of cimetidine upon the expected hepatic regeneration [11,12].

Antioxidants are capable of stabilize, deactivate or scavenge free radicals before they attack cells [13]. Antioxidants have shown beneficial effects, specifically for prevention and treatment of chronic liver injury [14-16]. Vitamin E is a potent antioxidant that provides hepatoprotection by scavenging free radicals [17]. Reports suggested that tocopherol reduced the hepatotoxicity of antitubercular drugs in animals [18-19]. Ranitidine possess hepatoprotective activity against galactosamine and CCl_4 induced hepatotoxicity [20] while vitamin E shows hepatoprotective activity against CCl_4 [21], monosodium glutamate [22], cadmium [23], thioacetamide [24], d-galactosamine [25] and halothane [26] induced liver injury in wistar rats.

Ranitidine and vitamin E, however, are less effective in the treatment of liver injury caused by severe oxidative stress when used alone [10,27,28]. The aim of the present study was to determine whether ranitidine and vitamin E might be additive in their protection against rifampicin hepatotoxicity.

Materials and methods

Drugs and Chemicals

Ranitidine [Ciron Drugs, Thane], vitamin E [E. Merck, Mumbai], rifampicin [Sigma Aldrich Pvt. Ltd. Bangalore] and silymarin [Micro Labs, Tamil Nadu, India], 5,5'-dithio bis-2-nitrobenzoic acid [DTNB or Ellman's reagent] [Research-Lab Fine Chem Industries, Mumbai], tris buffer [Sigma Aldrich Pvt. Ltd, Mumbai], pyrogallol [Loba chemie Pvt. Ltd. Mumbai] were procured. All the biochemicals and chemicals used were of analytical grade. Rifampicin [50 mg/kg] was dissolved in distilled water [1ml/kg] and then pH was adjusted to 3.0 with 0.1 N HCl to have a clear solution [29]. Ranitidine [50, 75, 100 and 120 mg/kg] was dissolved in distilled water [1ml/kg] orally administered to rats. Vitamin E [50, 75 and 100 mg/kg] was suspended in carboxymethylcellulose [1% CMC] orally administered to rats.

Animals

Male albino rats [four weak] of either sex weighing 180±20g were used for the study. They were housed in specific standard laboratory conditions in the animal house of pharmacology department, Appasaheb Birnale College of Pharmacy, Sangli and were kept at temperature 25±20^{0}C control environment, in a relative humidity 55-62%, with regular 12h light/12h dark cycle. All animals were fed with standard rat chow diet [Amrut Laboratories animal feed, Sangli, India], and water ad libitum. The experiment was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA], New Delhi, India and approved by the Institutional Animal Ethics Committee [IAEC].

Experimental design

Animals were divided into 21 groups [5 animals in each group]. Treatment duration was 4 weeks [30]. The treatments of Ranitidine [9,10] alone [50 mg/kg, 75 mg/kg, 100 mg/kg and 120 mg/kg body weight/day], vitamin E alone [50 mg/kg, 75 mg/kg and 100 mg/kg [31] [body weight/day], Silymarin [32] [100 mg/kg body weight/day] and ranitidine was given in combination with vitamin E were given 30 minutes prior to the administration of rifampicin.

Estimation of serum marker enzymes

Animals were sacrificed after last dose of rifampicin. Blood was collected, allowed to clot and the serum was separated. The blood was obtained from animals puncturing retro-orbital plexus. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm for 15 min at 30^{0}C and utilized for estimation of AST and ALT [33], ALP [34] and direct as well as total bilirubin content [35].

Antioxidant Activity

Preparation of 10% liver homogenate

After collection of blood samples, the rats were fasted for 12 h before sacrificed by cervical dislocation under light anesthesia. The liver was dissected out and used for estimation of LPO, SOD and glutathione activity. The livers were excised, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl [pH 7.4]. A 10% w/v of homogenate [36] was prepared in 0.15 M Tris-HCl buffer.

Measurement of antioxidant activities

The homogenate was used for the determination of lipid peroxidation [37], glutathione [38]. The rest of homogenate was centrifuged at 1500 rpm for 15 min at 4^0C. The supernatant [36] thus obtained was used for the estimation of superoxide dismutase [39 Marklund, 1985] and total protein [40].

Measurement of cytochrome P-450 activity

Preparation and isolation of liver microsomes

20-25% liver homogenate of fasted adult rats of body weight 400-500 g in homogenizer was prepared. The homogenizing medium consisted of isotonic sucrose containing 0.05 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, 0.025 M KC1 and 0.008 M $CaCl_2$. The pH 7.5 of the salt solutions was adjusted. After clarifying the homogenate at 10,000 g for 15 min, 4 or 5 ml [equivalent to 1 g wet weight tissue] of the post-mitochondrial supernatant was diluted with 25 ml of 0.0125 M sucrose solution containing 0.008 M $CaCl_2$, and 0.005 M $MgCl_2$. The entire solution was stirred occasionally for a few minutes and then centrifuged at 1500 g, for 10 min in a rotor. The reddish pellet obtained was dispersed and recentrifuged twice at 1500 g max for 10 min in dilute [0.0125 M] sucrose and finally homogenized in 0.05 M Tris-HCl buffer containing 0.005 $MgCl_2$ and 0.025 M KCl. Treating the rat liver under identical conditions described above with the exception of the addition of calcium chloride resulted in no pellet sedimenting at 1500 g for 10 min. The microsomal pellet was successively washed [rehomogenizing and sedimenting] twice in the same buffer and then finally suspended in the buffer. All operations for both preparations were carried out between 0-4 ^{0}C. Fresh samples were used in enzymatic assays [41]

Analysis of microsomal content of cytochrome P-450

Microsomes were suspended in 0.1 M phosphate buffer, pH 7.0 and reduced by a few mg of $Na_2S_2O_4$. The cytochrome P-450 content was measured [42, 43] in Jasco-550 UV-visible spectrophotometer and taking absorbance at 450 nm with cuvettes of 1-cm optical path. The conversion between 'g' value and rpm was calculated by following formula $G = [1.118 \times 10^{-5}] RS^2$.

Statistical analysis

Values are expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance [ANOVA] followed by using Dunnet's test, Newman-Keuls test and Tukey-Kramer multiple comparison test.

Results

In the present study, the administration of RMP [50 mg/kg body weight, orally] showed severe liver injury demonstrated by significant increases in serum marker enzymes as well as increase in level of lipid peroxidation in the liver of rat accompanied by a depletion of hepatic GSH and decrease in levels of SOD.

Serum AST, ALT, ALP and Bilirubin activities

Table 1 shows the levels of serum marker enzymes [AST, ALT, ALP, and bilirubin] in the serum of normal and experimental groups of rats. As shown in Table 1, animals treated with RMP showed a significant increase [$p<0.01$] in serum biomarker enzymes [AST, ALT, ALP, and bilirubin activities] as compared to normal control rats. But when rats were treated with different combinations of ranitidine plus vitamin E, levels of these enzymes were brought back to near normal status at a significant level [$p<0.01$]. In contrast, treatments with ranitidine alone or vitamin E alone slightly prevent the increases in these enzymes.

Table 1: Effect of different treatments of ranitidine and antioxidant vitamin E on serum marker enzyme levels in rifampicin induced hepatotoxicity

Treatments and dose (mg/kg)	ALT (SGPT) Units/ml	AST (SGOT) Units/ml	ALP KA Units	Bilirubin	
				Direct	Total
Normal Control (DW) 10 ml/kg	31.0 ±0.70	91.6±0.51	6.61±0.01	0.214±0.01	0.376±0.009
Rifampicin Control 50 mg/kg	101.8±1.15*	194.0±0.70*	27.3±0.08*	0.82±0.01*	1.6±0.0754*
Silymarin (Standard) 100 mg/kg	35.4±0.92**	92.8±0.86**	9.52±0.01**	0.262±0.012**	0.436±0.007**
120R	68.4±0.92**	109.0±0.70**	18.06±0.01**	0.57±0.01**	0.93±0.007**
100R	78.4±0.92**	135.8±0.66**	20.12±0.01**	0.620.007**	1.06±0.007**

75R	82.1±0.50**	143.6±0.50**	22.75±0.08**	0.73±0.007**	1.112±0.005**
50R	88.4±0.92**	185.2±0.86**	26.12±0.09**	0.778±0.008**	1.16±0.007**
100E	60.0±0.70**	110.0±0.70**	16.37±0.08**	0.54±0.01**	0.76±0.007**
80E	72.6±0.92**	141.0±0.70**	18.52±0.09**	0.58±0.007**	0.97±0.007**
60E	90.8±0.86**	177.8±0.86**	19.54±0.07**	0.616±0.009**	1.136±0.009**
40E	94.0±4.57**	184.8±0.86**	21.15±0.01**	0.758±0.007**	1.244±0.0132**
120R+100E	32.0±0.70**	97.0±0.70**	10.14±01.0**	0.23±0.007**	0.35±0.009**
100R+80E	33.0±0.70**	99.0±0.70**	11.37±0.08**	0.26±0.007**	0.43±0.013**
100R+60E	38.0±0.86**	103.8±0.86**	11.59±0.02**	0.40±0.007**	0.648±0.0106**
100R+40E	43.0±0.70**	111.4±1.03**	13.38±0.01**	0.31±0.007**	0.57±0.007**
75R+80E	40.2±0.86**	106.6±0.50**	12.10±0.01**	0.278±0.008**	0.546±0.013**
75R+60E	41.4±1.03**	115.2±0.86**	10.81±0.06**	0.346±0.009**	0.828±0.008**
75R+40E	65.6±0.92**	138.8±0.86**	22.84±0.07**	0.720±0.006**	0.950±0.007**
50R+80E	35.8±0.66**	120.0±0.70**	15.50±0.07**	0.280±0.008**	0.652±0.0106**
50R+60E	67.2±0.86**	173.8±0.86**	23.75±0.06**	0.740±0.006**	1.082±0.013**
50R+40E	69.4±0.92**	177.6±0.92**	25.92±0.08**	0.766±0.009**	1.19±0.0125**

Where R= Ranitidine, E= Vitamin E, DW = Distilled water, R+E= Ranitidine plus Vitamin E
SGOT- Serum Glutamate Oxaloacetate Transaminase, SGPT- Serum Glutamate
Pyruvate Transaminase, ALP- Alkaline Phosphatase
Significance evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test versus control group
All above groups received rifampicin 50 mg/kg, per oral for 28 days except normal group.
Values are expressed as the mean ± SEM, for 5 animals.
P values: *P<0.01 compared with normal control group
**P<0.01 compared with rifampicin control group

Antioxidant Activities

Table 2 shows the levels of antioxidant enzymes [LPO, SOD and reduced GSH] in the liver tissue of normal and experimental groups of rats.

Table 2: Effect of different treatments of ranitidine and antioxidant vitamin E on antioxidant enzyme levels in rifampicin induced hepatotoxicity

Treatments and dose (mg/kg)	LPO nmoles/mg protein	SOD Units/mg protein	GSH µmoles/mg protein
Normal Control (DW) 10 ml/kg	94.98±0.009	24.05±0.025	91.15±0.012
Rifampicin Control 50 mg/kg	292.64±0.008**	6.57±0.0092**	29.34±0.014**
Silymarin (Standard) 100 mg/kg	95.44±0.011***	21.23±0.009***	73.55±0.015***
120R	110.08±0.012***	14.00±0.010***	44.04±0.011***
100R	123.04±0.013***	13.42±0.010***	42.80±0.019***
75R	125.47±0.009***	12.36±0.012***	39.91±0.012***
50R	131.75±0.012***	10.95±0.013***	37.10±0.013***
100E	117.56±0.010***	12.12±0.010***	38.93±0.011***
80E	126.06±0.007***	11.95±0.013***	34.11±0.014***
60E	134.86±0.052***	10.77±0.015***	31.17±0.014***
40E	141.72±0.080***	8.53±0.007***	48.13±0.009***
120R+100E	96.67±0.011***	21.18±0s.018***	71.34±0.012***
100R+80E	97.14±0.014***	21.00±0.010***	69.96±0.014***
100R+60E	99.93±0.013***	19.83±0.010***	66.95±0.014***
100R+40E	101.53±0..031***	18.42±0.019***	63.28±0.010***
75R+80E	98.75±0.015***	19.63±0.017***	61.06±0.009***
75R+60E	100.12±0.016***	16.28±0.010***	69.77±0.011***
75R+40E	118.33±0.016***	17.77±0.008***	39.08±0.010***
50R+80E	102.85±0.017***	14.02±0.008***	60.85±0.013***
50R+60E	117.17±0.020***	17.25±0.074***	35.65±0.012***
50R+40E	120.64±0.128***	18.87±0.008***	30.55±0.015***

Where R= Ranitidine, E= Vitamin E, DW = Distilled water. R+E= Ranitidine plus Vitamin E, LPO-Lipid Peroxidation, SOD-Superoxide Dismutase, GSH-Glutathione.
Significance evaluated by one-way analysis of variance (ANOVA) followed by Newman-Keul multiple comparison t-test versus control group.
All above groups received rifampicin 50 mg/kg, per oral for 28 days except normal group.
Values are expressed as the mean ± SEM, for 5 animals
P values: **P<0.01 compared with normal control group
* **P<0.01 compared with rifampicin control group

Lipid peroxidation

Lipid peroxidation [LPO] was significantly [P<0.001] enhanced in animals exposed to RMP compared to the normal control group and this value was significantly decreased with the different combinations of ranitidine plus vitamin E.

Superoxide dismutase [SOD]

Superoxide dismutase was decreased significantly [P<0.001] in RMP-treated animals compared to normal group. Various supplementations of ranitidine plus vitamin E to RMP-treated animals prevented the lowering of superoxide dismutase level.

Reduced glutathione [GSH]

Reduced glutathione was decreased significantly [P<0.001] in RMP-treated animals compared to normal group. The additive combinations of ranitidine plus vitamin E to RMP-treated animals were maintained to near normalcy at a significant level [P<0.001].

Cytochrome P-450 activity

As shown in Table 3, cytochrome P-450 content was significantly [P<0.001] enhanced in animals exposed to RMP compared to the normal rats. These increases were prevented when rats were treated with ranitidine + vitamin E and maintained these alterations to the normal status with the different combinations of ranitidine plus vitamin E.
Treatment with ranitidine alone or vitamin E alone slightly prevent the increase in the levels of AST, ALT, ALP, Bilirubin, LPO, cytochrome P-450 content and decrease in the levels of reduced GSH and SOD in rifampicin hepatotoxicity.

Table 3: Effect of different treatments of ranitidine and antioxidant vitamin E on cytochrome P-450 content in rifampicin induced hepatotoxicity

Treatments and dose (mg/kg)	CYP-450 content (µmoles/mg protein)
Normal Control (DW) 10 ml/kg	0.22±0.018
Rifampicin Control 50 mg/kg	0.83±0.008**
Standard (Silymarin) 100 mg/kg	0.24±0.006***
120R	0.45±0.010***
100R	0.49±0.009***
75R	0.51±0.009***
50R	0.57±0.007***
100E	0.43±0.005***
80E	0.50±0.007***
60E	0.52±0.006***
40E	0.59±0.005***
120R+100E	0.25±0.011***
100R+80E	0.28±0.01***
100R+60E	0.31±0.007***
100R+40E	0.33±0.006***

75R+80E	0.29±0.007***
75R+60E	0.34±0.005***
75R+40E	0.50±0.005***
50R+80E	0.32±0.015***
50R+60E	0.51±0.009***
50R+40E	0.54±0.01***

Where, CYP-450= Cytochrome P-450, DW= Distilled Water, R= Ranitidine, E= Vitamin E
Significance evaluated by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test versus control group.
All above groups received rifampicin 50 mg/kg, per oral for 28 days except normal group.
Values are expressed as the mean ± SEM, for 5 animals.
P values: **P<0.01 compared with normal control group
* **P<0.01 compared with rifampicin control group

1. Discussion

Rat model was used to study the hepatotoxic effect of RMP and additive hepatoprotective effect of ranitidine and vitamin E. Rats have been successfully used to establish RMP-induced hepatotoxicity models. In the present study, hepatotoxicity was produced by RMP at a dose of 50 mg/kg body weight/day for four weeks. The dose is very high compared to that in the treatment of tuberculosis in human subjects, because higher doses are required for animals like rats as they metabolize the drugs at a faster rate [44]. To the best of our knowledge, the present study is to provide data to suggest a significant additive antioxidative and liver protective effect for these two compounds.

It was evidenced that biotransformation of rifampicin into its active metabolite, 25-desacetyl rifampicin reduces the drug metabolizing enzymes and specifically binds to RNA polymerase which inhibits the nucleic acid and protein synthesis responsible for hepatotoxicity [45].

Assessments of liver toxicity was done by measuring the marker enzymes viz AST, ALT, ALP and LDH, which are originally present in high concentration in the cytoplasm. Elevated levels of serum enzymes like AST, ALT, ALP and Bilirubin are indicative of cellular leakage and loss of functional integrity of cell membrane in liver [46]. When there is hepatic injury these enzymes leak into blood stream inconformity with extent of hepatoxicity. In present study, the elevated levels of serum marker enzymes like AST, ALT, ALP and bilirubin after RMP exposure. The values are reversed to normal after combined treatment of ranitidine plus vitamin E than ranitidine alone or vitamin E alone. This may be result of stabilization of plasma membrane as well as the repair of hepatic tissue injury caused by rifampicin.

LPO modifies the structure of biomembranes and the activity of liver drug-metabolizing enzymes and especially cytochrome P-450 dependent microsomal monoxygenases [47]. MDA is one of the end products in the lipid per-oxidation process [36]. Margarita K showed that histamine H_2-receptor antagonists are able to prevent gastric mucosal lipid peroxidation [7]. Also it was reported that main antioxidant function of vitamin E is protection against lipid peroxidation [48]. Vitamin E is a natural antioxidant and extremely effective in

detoxifying harmful free radicals in various tissues. It has been shown to inhibit hepatocyte lipid peroxidation caused by free radical forming agents like carbon tetrachloride [49] and halothane [26]. In the present study, free radicals formed either by the reaction of drug's radicals with oxygen or by the interaction of superoxide radicals with hydrogen peroxide seemed to initiate lipid peroxidation in RMP-treated rats, suggesting that increased lipid peroxidation might be associated with cellular damage. Ranitidine combined with vitamin E prevented significantly the peroxidation of lipids in animals exposed to RMP either directly or through non-protein thiols [GSH] by scavenging the radicals.

GSH is one of the most abundant tripeptide non-enzymatic intracellular biological antioxidant [50] present in liver. It is involved in the removal of free radicals such as H_2O_2, superoxide anions and alkoxy radicals, preserving membrane protein thiols and a substrate for glutathione peroxidase and glutathione reductase protein thiols and a substrate for glutathione peroxidase and glutathione reductase. In the present experiments, rifampicin treatment increased the activities of GSH related enzymes thereby decreasing the GSH content in liver, whereas combined treatment with ranitidine and vitamin E able to reverse such effects. It may be understood that effect of ranitidine and vitamin E may be due to an initial reduction in hepatic peroxidative activities, followed by inhibition of the activities of GSH related enzymes. A fore mentioned effects with ranitidine and vitamin E treatment finally results into restoration of reduced GSH content rifampicin induced hepatotoxicity.

Superoxide dismutase [SOD] is a key defense enzyme and catalyzes the dismutation of superoxide anions. Decreased in SOD activity can result in the decreased removal of superoxide anions that may inactivate SOD thereby causing an inactivation of H_2O_2 scavenging enzymes [51]. It is quite likely that administration of ranitidine plus vitamin E to rifampicin treated rats able to prevent effectively the decrease in SOD.

Cytochrome P-450 mediates generation of reactive metabolites of drugs and their covalent binding to hepatic macromolecules is the most accepted mechanism of RMP-induced hepatic injury [52]. RMP is a potent inducer of cytochrome P-450 [53]. Supplementation of ranitidine plus vitamin E to RMP-treated and control animals had significant effect on cytochrome P450 in the present study.

The three major hypotheses can be proposed to explain the mechanisms for the additive hepatoprotective effects provided by the combined treatment of ranitidine E plus vitamin E.

First, the specific chemical property of these two compounds enabling them to simultaneously scavenge the overproduced free radicals following rifampicin insult from various tissue and cellular components. All two compounds i.e. ranitidine [6] and vitamin E [58] play an important and different role in scavenging free radicals. Vitamin E or vitamin C alone or in combination can be given as prophylactic/therapeutic supplements for combating scavenging free radicals generated in liver tissue [54].

Second, the interaction among between ranitidine and vitamin E may maintain them in their reduced form to rebuild the antioxidant balances that could have been impaired by elevated oxidative stress following RMP attack. Reported by Hagymasi and Biazovics, [55] that combined antioxidant treatment was more favourable compared with monotherapy. Also some favourable results regarding with combined antioxidant therapy of liver disease of

different etiology have been reported. Vitamin E can work synergistically with other supplements in protecting against liver injury [54,56]. These compounds, however, are less effective in the treatment of liver injury caused by severe oxidative stress when used alone [10, 27,28]. Hence we would achieve additive effect against RMP hepatotoxicity.

Third, additive protection of ranitidine and vitamin E in RMP hepatic injury may due to its capacity to bind cytochrome P-450. Like similar pattern, the protective effect of ranitidine observed in this animal due to its capacity to bind cytochrome P-450 and prevent the oxidation of acetaminophen to its toxic metabolites [9]. We presume that ranitidine binds liver microsomes and inhibits the activity of P-450 in spite of lack of an imidazole base [57]. Also it was reported that the stabilizing effect of vitamin E on cytochrome P-450 is dose dependent [47].

Since antioxidants directly target free radicals formed during INH and RMP metabolism which are ultimately responsible for liver cell necrosis, this might explain superior efficacy of vitamin E and ranitidine [cytochrome P-450 inhibitor] which acts at an earlier step in [INH] isoniazide and RMP metabolism pathway. Based on this background, we hypothesized that the combined antioxidative treatment with ranitidine and vitamin E would achieve a significant additive hepatoprotective effect, thus greatly protecting the liver from injury induced by oxidative stress.

We have shown in the present study that, treatment with ranitidine alone or vitamin E alone slightly improved serum marker enzymes, antioxidant activities and cytochrome P-450 content in dose related manner but the protection by the combination of ranitidine and vitamin E was additive when compared to each agent alone. The results revealed that combined treatment of ranitidine plus vitamin E greatly decreased rifampicin-induced hepatotoxicity.

From the present study, it can be concluded that the additive hepatoprotective effect of ranitidine plus vitamin E may be due to the radicals scavenged by ranitidine as well as vitamin E. The mechanism may be related to the additive antioxidative effect among these two compounds. At the present stage, we presume that the inhibition of cytochrome P-450 is the main factor in the protection by ranitidine and vitamin E against rifampicin induced hepatic injury.

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Abbreviations

RMP: Rifampicin, R: Ranitidine, E: Vitamin E, R+E: Ranitidine plus vitamin E, AST:Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline posphatase, LPO: Lipid peroxidation, SOD: Superoxide dismutase, GSH: Reduced glutathione, G: the relative centrifugal force, R: the radius of the rotor [cm], S: the speed of the centrifuge [revolutions per minutes i.e. rpm]