Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2020.026

RESEARCH ARTICLE

Vitamin E and Selenium Attenuate Hepatotoxicity, Nephrotoxicity and Oxidative Stress Induced by Rifampicin in Rabbits

Amina Naseer[1§], Asif Hussain[1§], Bilal Aslam[1*], Faqir Muhammad[1], Mashkoor Mohsin[2], Muhammad Usman Bari[1], Aisha Sultana[1] and Anum Masood[3]

[1]Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
[2]Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Biochemistry, Faculty of Science, University of Agriculture, Faisalabad, Pakistan
*Corresponding author: cba933@gmail.com

ARTICLE HISTORY (19-467)

Received: October 13, 2019
Revised: December 05, 2019
Accepted: December 15, 2019
Published online: March 11, 2020

Key words:
Hepatoxicity
Nephrotoxicity
Oxidative stress
Rifampicin
Selenium
Vitamin E

ABSTRACT

Rifampicin (RIF) is a first-line anti-tuberculosis drug, besides beneficial effects, it triggers hepatic and renal toxicities. Drug toxicity is assumed to be associated with increased cellular oxidative stress. In the present study, the protective effect of vitamin E (α-tocopherol) and selenium (Se) on hepatic function biomarkers and alteration in renal function against rifampicin-induced toxicity was assessed. Rabbits were selected and placed into six groups (n=6). For 14 days experiment, RIF (100 mg/kg/day) was given to induce hepatorenal toxicity in all animals except normal control. Silymarin (200 mg/kg) was given as standard protective drug. Three groups received Vit. E (50 mg/kg), Se (1 mg/kg) and combination of both, respectively. Blood samples were collected to check hepatic function (ALT, AST, ALP), renal function (creatinine, urea), oxidative stress biomarkers (TOS, TAC, MDA, catalase), and liver and kidney tissue samples for histopathological analysis. Vit. E and Se exhibited significant ($P \leq 0.05$) protective effect by ameliorating serum levels of ALT, AST, ALP, urea, creatinine against rifampicin induced liver and kidney injury. The protective effect is also evidenced by hepatorenal histopathological analysis. Moreover, elevated oxidative stress accompanied by rifampicin exposure was significantly restored as Vit. E and Se use elevated the serum levels of catalase and TAC, and reduced MDA and TOS. However, Vit. E and Se in combination exhibit a more significant protective effect than alone. Vit. E and Se have therapeutic application in rifampicin associated hepatorenal toxicity presumably through antioxidant properties.

To Cite This Article: Naseer A, Hussain A, Aslam B, Muhammad F, Mohsin M, Bari MU, Sultana A and Masood A, 2020. Vitamin e and selenium attenuate hepatotoxicity, nephrotoxicity and oxidative stress induced by rifampicin in rabbits. Pak Vet J, 40(3): 277-282. http://dx.doi.org/10.29261/pakvetj/2020.026

INTRODUCTION

Liver is a vital organ of the body which performs significant metabolic activities. Hepatic parenchyma majorly comprised of hepatocytes which are responsible for maintaining normal body physiological functions. It regulates carbohydrates, fats, proteins and vitamin metabolism. Liver tissues have the capacity to regenerate rapidly (Beckwitt *et al.,* 2018). However, various factors including hepatotoxic drugs, environmental toxins and hepatitis cause hepatotoxicity while chronic liver disease is caused by viral disease and excessive alcohol consumption (Woolbright and Jaeschke, 2018). Hepatic diseases cause greater morbidity and mortality worldwide. Liver disease burden is increased which has a significant impact on the overall world population (Asrani *et al.,* 2019). Kidney plays a major role in body homeostasis, excretion of detoxifying metabolites and drugs from the body. Therefore, direct exposure to exogenous toxic agents causes damage to the kidney. In renal toxicity, the excretory system becomes impaired which causes accumulation of these toxic chemicals in the body. Approximately 20% of renal toxicity is drug-induced while it increases up to 66% in elderly patients. Chemotherapy-associated renal toxicity is a major concern in cancer patients (Luyckx *et al.,* 2018).

§These authors contributed equally to this work.

Hepatorenal diseases account the major morbidity and mortality and hinder the effectiveness of various new drugs. Synthetic drugs exhibit unavoidable side effects which raised the hepatorenal toxicity risk. Lake of proper diagnosis, observation and underreporting makes it difficult to its definite prevalence (Mindikoglu and Stephen, 2018). Rifampicin (RIF) is one of first-line anti-tuberculosis drug which is effective against infectious agent *Mycobacterium tuberculosis.* It is estimated that tuberculosis affected approximately 1/3rd of the world population (Maltempe *et al.,* 2017). Recommended therapy includes a combination of isoniazid, pyrazinamide and rifampicin for six months, but serious adverse effects such as hepatotoxicity and nephrotoxicity associated with the use of these drugs are a major concern. Studies showed that rifampicin induced hepatitis cases increased in TB patients (Baskaran and Evan, 2017). Free radicals generated during oxidative reactions at the cellular level are usually neutralized by defensive antioxidant mechanisms. The production of free radicals during normal metabolic activities and their accumulation greatly increased in reproducing cells (Pisoschi and Aneta, 2015).

Vitamin E acts as an antioxidant that scavenges lipid peroxides and free reactive oxygen species intracellularly. It provides protection to oxidative stress and lipid peroxidation and contributes towards maintaining the cell membrane integrity (Galli *et al.,* 2017). Selenium (Se) acts as a cofactor of glutathione peroxidase enzyme which regulates the hydroperoxidase activity. Se is one of the essential trace elements which acts as an antioxidant in the living body (Li *et al.,* 2015). Earlier studies reported selenium physiological role as a growth promoter, anticancer and regulation of immune response. It protects body cells from the damaging effects of oxidative reactions generated free radicals (Khurana *et al.,* 2019). Therefore, this research was planned to investigate the protective effect of Vit. E and Se against liver and kidney injury induced by rifampicin.

MATERIALS AND METHODS

Animals: Thirty-six adult rabbits (1300-1500 gram) were bred and housed in the animal house of the Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan. Animals were kept in individual well-ventilated cages and acclimatized at 25±3ºC temperature, a light/dark cycle of 12-h and relative humidity maintained (40-60%), provided with seasonal fodder and clean water *ad-libitum*. Animals were acclimatized for one week before start of experiment.

The study was approved by Directorate of Graduate Studies, University of Agriculture, Faisalabad, Pakistan (DGS No. 2845-48). Animals were treated and cared according to the Guide for the Care and Use of Laboratory Animals. This study did not involve any human data, so consent to participate is not applicable.

Chemicals: Rifampicin (Pacific Pharmaceuticals Ltd, Lahore, Pakistan), silymarin (Abbott Lab., Karachi, Pakistan), Vitamin E (Merck Pharmaceuticals Pvt. Ltd., Karachi-Pakistan) and selenium as sodium selenite (Asia pharmaceuticals Pvt. Ltd. Faisalabad-Pakistan) were purchased. All other reagents of analytical grade were purchased locally and used in this study.

Experimental design: A total number of 36 albino rabbits of either sex was randomly selected and grouped into 6 groups (n=6). Group I on normal saline was used as normal control (NC). Rifampicin (100 mg/kg/day, p.o.) was given to the other 5 groups for hepatic and renal toxicities induction. Group II used as Positive control and Group III (SIL) received silymarin (200 mg/kg/day, p.o.). Group IV to VI received Vit. E (50 mg/kg/day, p.o.), Se (1 mg/kg/day, p.o.) and combination of Vit. E and Se for two weeks, respectively.

Biochemical analysis: On the 15th day of the experiment, animals were euthanized by cervical dislocation under mild anesthesia. Blood samples were collected in non-heparinized tubes, kept at room temperature for 30 mins and then centrifuged at 1010 x g for 10 min. Separated serum samples were stored at -20ºC in the biomedical freezer until further analysis. Biochemical parameters such as ALT, AST, ALP, creatinine and urea were estimated with commercially available kits of Merck Pvt., Ltd., Pakistan and analyzed by using automated serum analyzer (Bio-Ray 310 diagnostic). Oxidative stress parameters were determined by measuring serum levels of total oxidant status (TOS), total antioxidant capacity (TAC), malonaldehyde (MDA) and catalase (CAT) activity using microplate spectrophotometer (Thermo Scientific Multiskan Go^{TM} with SkanIt software 4.1) by following previously described methods.

Histopathological analysis: Liver and kidney samples were collected, washed with normal saline and stored in neutral buffered formalin solution (10% v/v) for histopathological examination. Tissues then fixed, embedded in paraffin and sections of 5 µm thickness were prepared with a rotary microtome. Hematoxylin and eosin (H&E) dyes were used for staining. Prepared tissue slides were examined with a camera (TOUPCAM, ToupTek Photonics Co., Ltd., China) attached to an automatic light microscope (Olympus PM–10ADS, Olympus Optical Co., Tokyo, Japan). The liver was examined for cellular infiltration, pyknotic nuclei, cytoplasmic vacuolation, necrosis and sinusoidal dilatation. Kidney tissues were analyzed for histopathological manifestations include cellular infiltrations, hemorrhage, cytoplasmic vacuolation, membrane thickening, necrosis and pyknotic nuclei (Bancroft and Gamble, 2007).

Statistical analysis: All collected data were analyzed by applying the one-way analysis of variance (ANOVA) and post-hoc Tukey's test, with a difference ($P \leq 0.05$) considered significant by using Prism 6 statistical package program (San Diego, CA, USA). All results were presents as Mean ± SD.

RESULTS

RIF administration induced hepatorenal toxicity in experimental animals: After 14 days experiment, results showed a significant ($P \leq 0.001$) elevation in liver drug/ metabolizing enzymes include alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), on rifampicin exposure (Fig. 1). Serum creatinine and urea levels were measured for renal

function evaluation. RIF administration significantly (P≤0.001) increased serum creatinine and urea levels after 14 days (Fig. 2). These results depicted that RIF produced a significant elevation in serum hepatorenal function biomarkers.

Vit. E and Se ameliorating effect on RIF administered acute hepatorenal injury: Biochemical analysis showed that coadministration of Vit. E and Se prevented RIF induced hepatic tissue damage, which also evident by histopathological analysis. Vit. E and Se treated groups indicated significant (P≤0.05) reduction in serum transaminases levels accompanied by normalization of hepatic tissue architecture (Fig. 1). Renal biomarkers showed a preventive effect of Vit. E and Se coadministration with rifampicin (Fig. 2). However, Vit. E and Se produced a more significant preventive effect in combination than alone.

Restoration of antioxidant capacity by Vit. E and Se against RIF administration: RIF administration showed an increase in oxidative stress, while a reduction in total antioxidant capacity. Malonaldehyde level was significantly elevated and reduction in catalase level accompanied by RIF exposure was observed (Fig. 3). Administration of Vit. E and Se and in combination produced significant (P≤0.05) protective effect against RIF induced oxidative stress. Vit. E and Se showed significant (P≤0.05) reduction of serum total oxidant status and malonaldehyde levels as compared to RIF treated group. Vit. E and Se significantly (P≤0.05) increased total antioxidant capacity and catalase level, and combination showed more profound effects against RIF induced oxidative stress.

Histopathological findings: Liver histopathological examination revealed normal morphology of hepatic tissue with regular parenchyma, sinusoids, vesicular structure with normal cytoplasmic appearance and absence of pathological alterations in the control group (Fig. 4). Morphological alterations including severe hepatic lesions, cytoplasmic vacuolation and hyper-chromasia were observed with RIF. Coadministration of rifampicin with concomitant use of Vit. E and Se significantly prevented histopathological changes and provide protection with reduced cytoplasmic vacuolation and well-formed polygonal hepatocytes, in comparison to RIF treated animals. In kidney specimens, the regular architecture of glomeruli, proximal tubules and nucleus was observed (Fig. 5). RIF treated animals showed severe renal tissue injury evidenced by renal blood vessels thickening and vacuolation, cell pyknosis and necrosis of renal tubular tissue, edema, tubular inflammation and infiltration of inflammatory cells. Animals treated with Vit. E and Se showed a relieved degree of renal lesions and tubular damage, normal renal parenchyma with reduced tubules inflammation, dilation and blood vessel congestion. Some renal sections showed almost normal glomeruli and tubules appearance with slight degenerative changes with Vit. E and Se treatment.

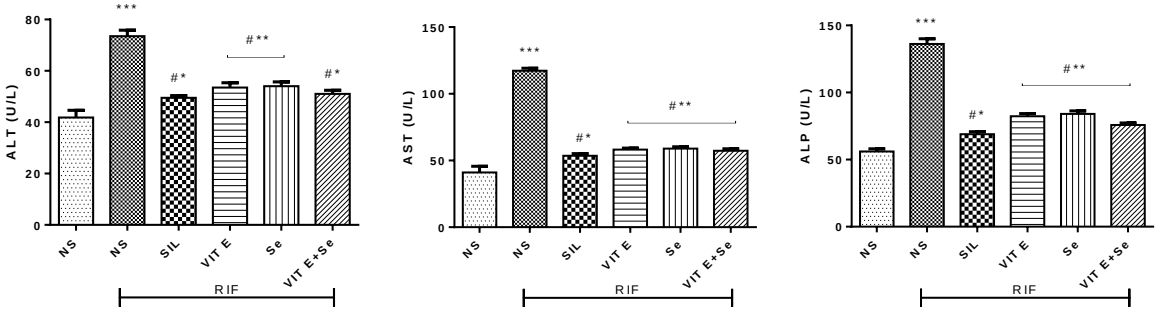

Fig. 1: Establishment of alterations in liver function enzymes on rifampicin administration. Rifampicin (100 mg/kg p.o.) induced acute liver toxicity in rabbits after 14 days of treatment. Serum levels of liver function enzymes ALT, AST and ALP were assessed to determine the protective effect of Vit E, Se and their combination against rifampicin induced acute live injury. *(P≤0.05), **(P≤0.01), ***(P≤0.001) shown a significant difference in treated groups from normal saline control. #(P≤0.05) shown a significant difference in treated animals from positive control group.

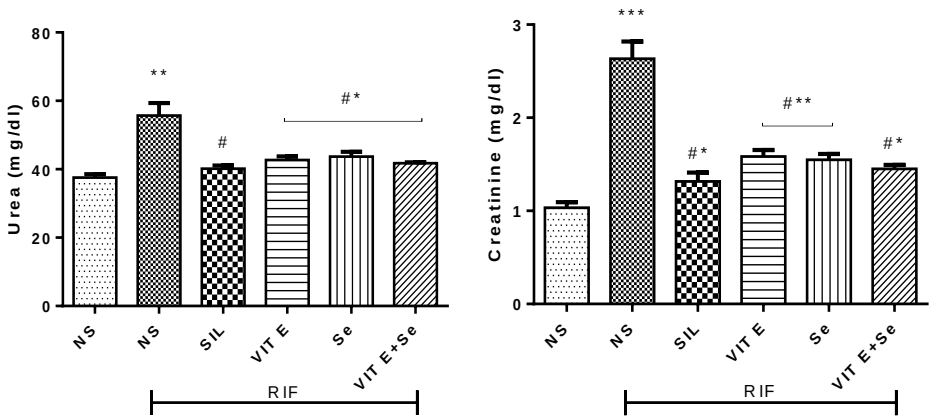

Fig. 2: Establishment of alterations in kidney function on rifampicin administration. Rifampicin (100 mg/kg p.o.) induced acute renal toxicity in rabbits after 14 days of treatment. Serum levels of renal function biomarkers including urea and creatinine were assessed to determine the protective effect of Vit E, Se and their combination against rifampicin induced acute renal injury. *(P≤0.05), **(P≤0.01), ***(P≤0.001) shown a significant difference in treated groups from normal saline control. #(P≤0.05) shown a significant difference in treated animals from positive control group.

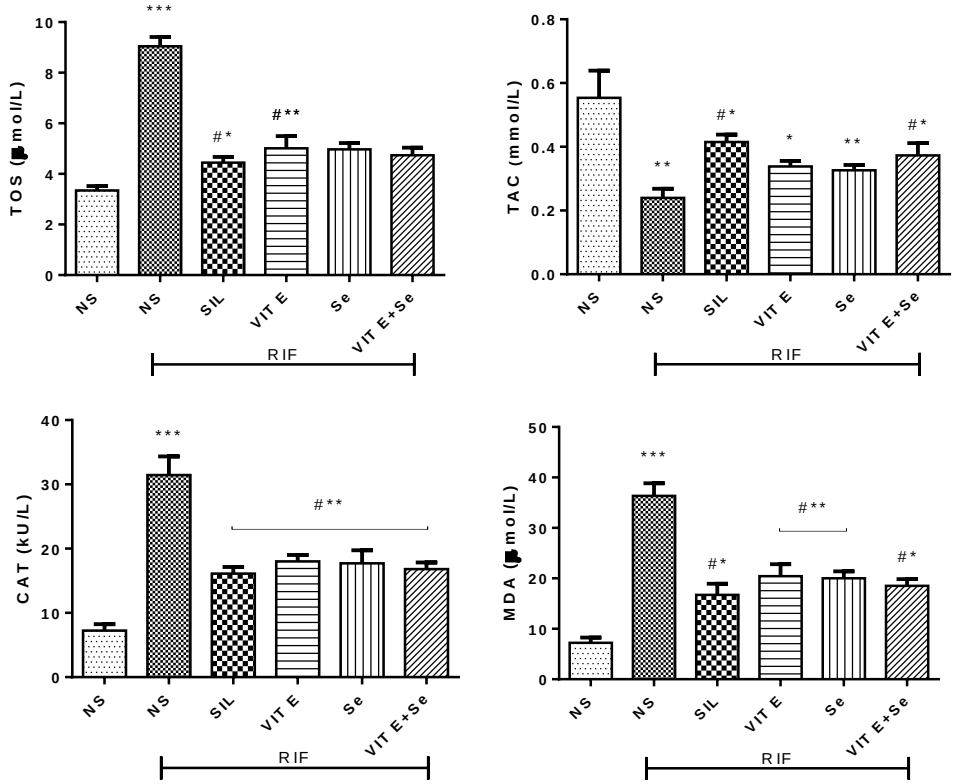

Fig. 3: Establishment of alterations in oxidative stress biomarkers on rifampicin administration. Rifampicin (100 mg/kg p.o.) induced oxidative stress in rabbits after 14 days of treatment. Serum levels of oxidative stress biomarkers include TOS, TAC, CAT and MDA activity were assessed to determine the protective effect of Vit E, Se and their combination against rifampicin treatment. *(P≤0.05), **(P≤0.01), ***(P≤0.001) shown a significant difference in treated groups from normal saline control. $^{\#}$(P≤0.05) shown a significant difference in treated animals from positive control group.

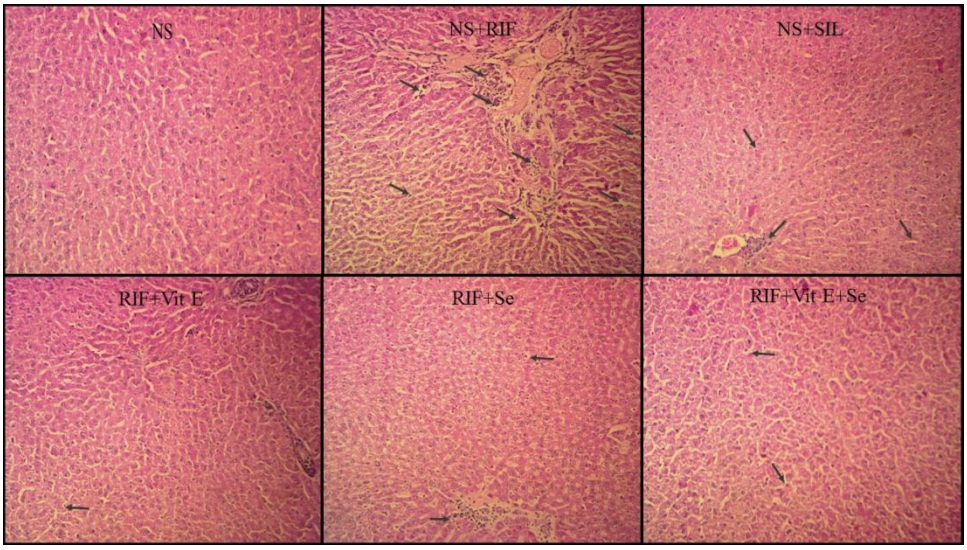

Fig. 4: Histopathological examination of liver tissue (NS) normal saline control (NS+RIF) rifampicin-induced liver damage, (NS+SIL) silymarin hepatoprotection on rifampicin exposure, (RIF+Vit E) Vit. E protective effect against rifampicin, (RIF+Se) Se treated group showed protection against rifampicin-induced liver injury, (RIF+Vit E+Se) Coadministration of Vit. E and Se showed protection with rifampicin treatment.

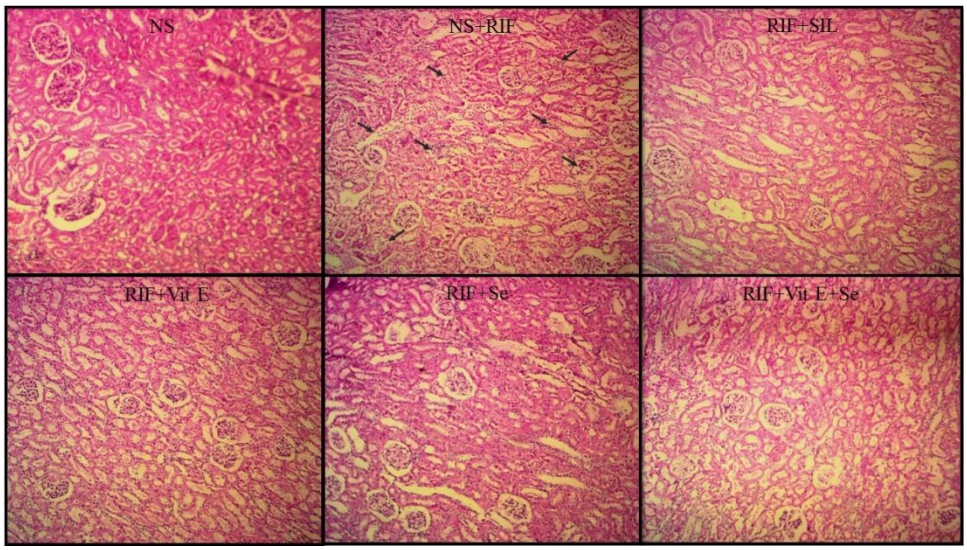

Fig. 5: Histopathological examination of renal tissue (NS) normal control, (NS+RIF) rifampicin-induced renal damage, (RIF+SIL) silymarin renal protection on rifampicin exposure, (RIF+Vit E) Vit. E protective effect against rifampicin, (RIF+Se) Se treated group showed protection against rifampicin-induced kidney injury, (RIF+Vit E+Se) Coadministration of Vit. E and Se showed protection with rifampicin treatment.

DISCUSSION

Tuberculosis (TB) is a communicable airborne disease. According to WHO, TB is ranked third infectious disease responsible for mortality worldwide. Recommended treatment strategies included the use of antibiotics combination therapy for six months. Long-term use of anti-tuberculosis drugs such as rifampicin is associated with serious side effects including hepatorenal toxicity (Glaziou *et al.*, 2013).

Overburden of reactive oxygen species generation during oxidative reactions is responsible for anti-tuberculosis drugs induced hepatorenal toxicity (Jeong *et al.*, 2015). Lipid peroxidation due to increased oxidative stress compromises the integrity of hepatocytes cell membrane (Li *et al.*, 2015). The deleterious effects of rifampicin were observed by increased serum levels of hepatic function enzymes (Nelson *et al.*, 2014). Elevation of liver enzymes activity is important in the assessment of liver diseases because increased lipid peroxidation is attributed to changes in hepatocytes cell membrane lipids organization, which results in leakage of cytoplasmic contents into main blood circulation due to increased cell membrane permeability (Swamy *et al.*, 2012). Previous studies showed renal function was affected in rifampicin-treated animals. Serum creatinine and urea levels were significantly enhanced which predict the compromised renal function (Beebe *et al.*, 2015).

In our study, anti-tuberculosis drug rifampicin induced hepatic and renal injury was noticed by the significantly elevated serum levels of hepatic function markers (ALT, AST, ALP) and renal function markers (creatinine, urea). The aim of this study was to assess the potential preventive effect of Vit. E and Se and their combination of rifampicin induced liver and kidney injury in rabbits.

Selenium (Se) is one of essential trace element for living organisms which involved in the regulation of antioxidant mechanisms and plays important role in the protection of macromolecules such as proteins and DNA against oxidative stress-induced cellular damage (Pfister *et al.*, 2016). Vit. E (α-tocopherol) is a fat-soluble vitamin present in vegetable oils. It plays a vital role in various physiological functions such as the stabilization of cell membrane integrity. Vit. E acts as an antioxidant by neutralizing the unstable reactive oxygen species and prevent the damaging effects of lipid peroxidation in tissue (Lebold and Maret, 2014).

In the current study, the administration of Vit. E at the dose of 50 mg/kg and selenium at 1 mg/kg exhibited significant preservation of hepatorenal activities against rifampicin exerted toxic effects due to their antioxidant potential.

Liver function enzymes including ALT and AST elevated levels in the blood indicate cellular damage. The increased levels of these enzymes were observed after rifampicin administration, which causes cell membrane structural damage and functional loss which results in increased cell membrane permeability and hepatic enzymes leakage into the blood circulation (Naik and Vandana, 2008). Alkaline phosphatase (ALP) is a cholestatic enzyme and its increased serum level indicates the cholestatic damage. ALP is present in the cellular lining of liver bile ducts. In hepatic diseases such as infiltrative diseases, intrahepatic cholestasis or bile duct obstruction, the level of ALP significantly increases. Similar hepatic damaging effects were previously reported (Kim *et al.*, 2017).

Kidney diseases result in either failure to excretion of waste products, creatinine and urea or failure to retain ions, water, amino acids and proteins (Lopez-Giacoman and Magdalena, 2015). In this study, rifampicin treatment showed increased serum urea and creatinine levels as compared to the control group, which indicates the failure to excrete these substances by the kidney. The results of this study showed that Vit. E and Se exhibited significant therapeutic effects on hepatic and renal function biomarkers that were increased following rifampicin exposure, which associated with oxidative process generated free radicals and hepatic and renal damage due to the unstable reactive oxygen species as observed in previous studies (Ozkol *et al.*, 2017; Mohamed *et al.*, 2019).

Body natural defense system includes immune cells and antioxidant mechanisms. Antioxidants protect from damaging effects of reactive oxygen species and free radicals generated during metabolic reactions (Ighodaro and Akinloye, 2018). Impaired defensive antioxidant system results upon exposure to free radicals, reactive oxygen and nitrogen species and toxin which greatly increases oxidative stress (Birben *et al.*, 2012). In our study, Vit. E and Se showed normalization of oxidative status parameters (TOS, TAC, MDA, Catalase activity) on the exposure of rifampicin. After observing the protective effect of Vit. E and Se on hepatorenal functioning, it is explored whether Vit. and Se exerted antioxidant potential as noticed by assessing serum TOS, TAC, MDA and catalase activities. Rifampicin administration increased the TOS and MDA levels while the reduction in TAC and catalase activity was observed. Administration of Vit. E and Se and their combination exhibited an ameliorating effect on concomitant rifampicin treatment.

Although the current investigation discloses the protective effects of Vit. E and Se against rifampicin induced hepatic and renal toxicities, the present work has some limitations. Further studies are required to ascertain molecular mechanisms involved, which might improve the understanding of pathophysiological aspects of liver and kidney diseases and improvement of therapeutic strategies.

Conclusions: We concluded that Vit. E and Se showed the hepatic and renal protective effect on rifampicin exposure by suppressing oxidative stress *in vivo* and ameliorating the biochemical as well as histopathological parameters in rabbits. Vit. E and Se have the tendency to ameliorate the severity of hepatorenal damage by rifampicin administration. It is persuasive to use Vit. E and Se in oxidative stress and drug-mediated toxicities.

Authors contribution: AN and BA designed the study. AH and MUB performed sampling and animal handling. AN and AM performed the biochemical analysis. AS and AH performed data acquisition and interpretations. AN and AH wrote the manuscript. FM and MM critically analyzed the data and approved the final manuscript. The authors

approved the manuscript and agreed to be accountable for liabilities pertaining to the content of this study.

Abbreviations: ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, CAT: catalase, MDA: malondialdehyde, TAC: total antioxidant capacity, TOS: total oxidant status, RIF: Rifampicin, Se: Selenium, SIL: Silymarin, Vit E: Vitamin E.

Acknowledgements: Authors are grateful to Sajid Masih, Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad-Pakistan for his help in animals handling during the experimental work.

REFERENCES

Asrani SK, Harshad D, John E, *et al*., 2019. Burden of liver diseases in the world. J Hepatol 70:151-71.

Bancroft JD and Gamble M, 2007. Theory and Practice of Histological Techniques. Philadelphia, Churchill Livingstone pp:131-3.

Baskaran UL and Evan PS, 2017. Clinical and experimental research in antituberculosis drug-induced hepatotoxicity: a review. J Integr Med 15:27-36.

Beebe A, Seaworth B and Naveen P, 2015. Rifampicin-induced nephrotoxicity in a tuberculosis patient. J Clin Tuberc Other Mycobact Dis 1:13-5.

Beckwitt CH, Amanda MC, Sarah W, *et al*., 2018. Liver 'organ on a chip'. Exp Cell Res 363:15-25.

Birben E, Umit MS, Cansin S, *et al*., 2012. Oxidative stress and antioxidant defense. World Allergy Organ J 5:9-19.

Galli F, Angelo A, Marc B, *et al*., 2017. Vitamin E: Emerging aspects and new directions. Free Radic Biol Med 102:16-36.

Glaziou P, Dennis F, Katherine F, *et al*., 2013. Global epidemiology of tuberculosis. Semin Respir Crit Care Med 39:271-85.

Ighodaro OM and Akinloye OA, 2018. First-line defense antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defense grid. Alexandria J Med 54:287-93.

Jeong I, Jong-Sun P, Young-Jae C, *et al*., 2015. Drug-induced hepatotoxicity of anti-tuberculosis drugs and their serum levels. J Korean Med Sci 30:167-72.

Khurana A, Sravani T, Mohd AS, *et al*., 2019. Therapeutic applications of selenium nanoparticles. Biomed Pharmacother 111:802-12.

Kim J, Woong SN, Sun JK, *et al*., 2017. Mechanism investigation of rifampicin-induced liver injury using comparative toxicoproteomics in mice. Int J Mol Sci 18:14-7.

Lebold KM and Maret GT, 2014. Interactions between alpha-tocopherol, polyunsaturated fatty acids and lipoxygenases during embryogenesis. Free Radic Biol Med 66:13-9.

Li S, Hor-Yue T, Ning W, *et al*., 2015. The role of oxidative stress and antioxidants in liver diseases. Int J Mol Sci 16:26087-124.

Lopez-Giacoman S and Magdalena M, 2015. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol 4:57-73.

Luyckx VA, Marcello T and John WS, 2018. The global burden of kidney disease and the sustainable development goals. Bull World Health Organ 96:414-22.

Maltempe FG, Katiany RCF, Renata CRA, 2017. Activity of rifampicin and linezolid combination in *Mycobacterium tuberculosis*. Tuberculosis 104:24-29.

Mindikoglu AL and Stephen CP, 2018. New developments in hepatorenal syndrome. Clin Gastroenterol Hepatol 16:162-77.

Mohamed MA, Lotfi A, Badr EE, *et al*., 2019. The ameliorative effects of ceftriaxone and vitamin E against cisplatin-induced nephrotoxicity. Environ Sci Pollut Res Int 26:15248-54.

Naik SR and Vandana SP, 2008. Hepatoprotective effect of *Ginkgoselect* phytosome® in rifampicin induced liver injury in rats: Evidence of antioxidant activity. Fitoterapia 79:439-45.

Nelson J, Saggau DD and Nielsen JS, 2014. Rifampicin induced hepatotoxicity during treatment for chronic central serious chorioretinopathy. Retin Cases Brief Rep 8:70-2.

Ozkol H, Gulay B and Ragip B, 2017. Protective effects of selenium, n-acetylcysteine and vitamin e against acute ethanol intoxication in rats. Biol Trace Elem Res 175:177-85.

Pfister C, Horst D and Franz-Josef S, 2016. Sodium selenite and cancer related lymphedema: Biological and pharmacological effects. J Trace Elem Med Biol 37:111-6.

Pisoschi AM and Aneta P, 2015. The role of antioxidants in the chemistry of oxidative stress: A review. Eur J Med Chem 97:55-74.

Swamy AH, Rucha RV, Koti BC *et al*., 2012. Hepatoprotective effect of *Cissus quadrangularis* stem extract against rifampicin-induced hepatotoxicity in rats. Indian J Pharm Sci 74:183-7.

Woolbright BL and Jaeschke H, 2018. Mechanisms of inflammatory liver injury and drug-induced hepatotoxicity. Curr Pharmacol Rep 4:346-57.