Received: 10 June 2023, Accepted: 15 August 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 7, 1394-1400

The Ameliorative Potential of Vitamin E and Selenium on the Possible Adverse Effects of Azithromycin

Ahmed S. Abdelaziz*, Zeinab A. Mabrouk, Hosny A. Ibrahim, Hesham A. Khalifa

Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44519, Egypt.

***Correspondence**
Corresponding author: Ahmed S. Abdelaziz
E-mail address: asabdelaziz@vet.zu.edu.eg
ORCID ID: 0000-0003-3004-0938

Abstract

Azithromycin, an antibiotic belonging to the macrolide group, is used not only for bacterial infections, but also for fungal and viral diseases. The main side effects of its use are hepatic and renal toxicities. The present study was carried out to evaluate the possible ameliorative effect of vitamin E and selenium against azithromycin-induced hepatic and renal toxicities. Fifty male albino rats used were allocated into 5 equal groups, each of 10 rats. All the treatments were taken orally for ten days in all groups. Group I served as a normal control. Group II received olive oil. Group III received azithromycin (6 mg/kg bwt). Groups IV and V received vitamin E and sodium selenite at doses of 100 mg/kg bwt and 0.3mg/kg bwt, respectively at two hours before azithromycin administration. Two blood samples were collected from each rat, one on heparin and the other without anticoagulant to obtain clear sera for hematological studies and biochemical analysis respectively. Liver and kidney tissues were collected for histopathological examination. Vitamin E or selenium administrations significantly decreased alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Gamma- glutamyle transferase (GGT) activities, creatinine, urea and uric acid levels, and MDA concentration in azithromycin-treated rats. Moreover, both Vitamin E and selenium significantly increased hematological parameters, protein profile (total protein, albumin, and globulin) and antioxidant enzyme activities (GPX, SOD, GSH, CAT) in rats treated with azithromycin with a significant improvement in the histopathological pictures of the hepatic and renal tissues. Based on these findings, vitamin E and selenium may be beneficial agents for protection against liver and kidney toxicities induced by azithromycin.

KEYWORDS
Azithromycin, Antioxidant status, Hepatic toxicity, Vitamin E, Selenium.

INTRODUCTION

Azithromycin (AZM), an antibiotic belonging to the macrolide group, is used since 1980 in routine clinical practice, seems to be a worthy drug to be used not only for bacterial infections, but also for fungal and viral diseases (Gabrie *et al*., 2021). Azithromycin is thought to increase the pH of Golgi network and recycling endosome, a fact that might interfere with the activity of SARS-CoV-2, jeopardizing its cycle within the cell. It is believed to possess a furin-like cleavage site in its spike protein (Poschet *et al*., 2020). In vitro studies showed that AZM can suppress the ZIKV infection and EBOV activity. In addition, it possesses anti-rhinoviral activity in bronchial epithelial cells and decreases RV replication. Moreover, recent studies showed that AZM had also anti-cancer activity (Khoshnood *et al*., 2022). Azithromycin, like other macrolide antimicrobials, binds to the 23S portion of the 50S bacterial ribosomal subunit and inhibits bacterial protein synthesis by preventing the transit of aminoacyl-tRNA and the growing protein through the ribosome. Compared to erythromycin, azithromycin is less prone to disassociation from the Gram-negative ribosome, conferring its greater efficacy against Gram-negative pathogens (Goldman *et al*., 1990).

The main adverse effects of azithromycin administration are renal and hepatic damages. The mechanism of toxicity is thought to be due to induction of oxidative stress and generation of free radicals leading to depletion of the antioxidant systems and membrane lipid peroxidation (Olayinka and Ore, 2014).

Vitamin E is a group of biologically active tocopherol widely distributed in plant products. The naturally occurring active compound is d- α tocopherol. It is a strong phenolic antioxidant, donating hydroxyl group on its ring structure to free radicals, preventing lipid peroxidation thereby prolonging the biological life of polyunsaturated fatty acids in the cell membranes by slowing the formation of free radicals and hyper-peroxides (Phillips *et al*., 1982; Mukai *et al*., 2007; Traber and Atkinson, 2007).

The protective roles of vitamin E against idarubicin induced myocardial toxicity (Kalender *et al*., 2002), heavy metal induced renal and testicular toxicity (Atef and Al-Attar, 2011), dimethoate induced cerebral toxicity (Amara *et al*., 2011) and chlorpyrifos toxicity in Atlantic salmons (Olsvik *et al*., 2015) were reported.

Selenium is a trace mineral that exists in minimal concentrations in the body but can play an important role in human health. Food sources high in selenium include Brazil nuts, seeds, mushrooms, fish, seafood, beef, and poultry meat (Shreenath *et al*., 2022).

Selenium serves as a cofactor for glutathione peroxidase and helps minimize oxidative damage through cellular metabolism (Tinggi, 2008). Selenium, in combination with vitamin E, protects

ISSN: 2090-6277/2090-6269/

cell membranes and organelles from peroxidative destruction (Shreenath *et al*., 2022).

A literature survey elucidated no scientific reports on the protective effect of Vitamin E and selenium against azithromycin-induced hepatic and renal toxicities. Therefore, in light of the above inferences from other evidence, we designed this study to investigate the beneficial role of Vitamin E and selenium against AZM-induced injuries to rat hepatic and renal tissues.

MATERIALS AND METHODS

Drugs

Azithromycin 100 mg/5 mL and powder of Vitamin E and sodium selenite were obtained from Egyphar Co., Obour city, Egypt.

Animals

Fifty male albino rats weighing 120-140 mg were obtained from laboratory animal's farm, Faculty of Veterinary Medicine, Zagazig University. The animals were housed in a room with controlled temperature (21-22°C), light (12 hour light dark cycle) and humidity (50±10%). The animals were allowed free access to standard laboratory food and water. The experiments were performed according to the guiding principles in the use of animals in toxicology and approved by the Institutional Animal Ethics Committee of Zagazig University (ZU-IACUC) under the number ZU-IACUC/2/F/144/2021.

Experimental design

Rats were allocated into 5 equal groups (10 rats each). Group I (control) was given orally 1 mL sterile distilled water to each rat. Group II (olive oil) received orally 1 Ml olive oil to each rat. Group III (azithromycin) received azithromycin at dose of 6 mg/kg bwt. Group IV (azithromycin + Vitamin E) received vitamin E at a dose of 100 mg/kg bwt two hours before azithromycin administration. Group V (azithromycin+selenium) received sodium selenite at 0.3mg/kg bwt two hours before azithromycin. All the treatments were given orally by stomach tube for ten days.

Blood and tissues samples

24 hours after the last treatment, the rats were sacrificed after light ether anesthesia by ketamine. We made every effort to comply with the strict guidelines to reduce pain and suffering. Then, two blood samples were collected from each rat. One sample was collected with anticoagulant (Heparin or EDTA) for hematological studies, and the other sample was collected without anticoagulant to allow separation of serum for biochemical analysis. Liver and kidney samples were taken in formalin for histopathological examination.

Haematological studies

Estimation of RBCs count, Hb content, WBCs count, PCV % and platelets count by using automated blood cells analyzer (Sysmex XT-2000 Iv, Kobe, Japan) (Buttarello and Plebani, 2008).

Estimation of liver enzymes levels

Serum levels of alanine transferases (ALT) and aspartate transferases (AST) were measured by methods described by Reitman and Frankel (1957) and the methods described by Kind and King (1954) were used to estimate serum levels of alkaline phosphatase (ALP) and (GGT).

Measurement of serum kidney function parameters

The methods described by Coulombe and Favreau (1963) were used in measurement of serum creatinine, urea, and uric acid, respectively.

Evaluation of antioxidant/oxidant status

For evaluation of lipid peroxidation, the method described by Uchiyama and Mihara (1978) was used to measure serum content of malondialdehyde (MDA). Tissue levels of reduced GSH, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) were determined according to the methods described by Draperi and Hadly, (1990).

Evaluation of protein profile

For evaluation of the protein profile, the method of Inoue *et al.* (2017) was followed.

Histopathological Examination

Using a rotatory microtome, 5 µm thick sections were sliced from the liver, and kidneys for histopathological examination. These sections were later stained with hematoxylin-eosin (H&E) dye (Merck) and examined at 200x magnification using a power light microscope (Zeiss, Germany). We used a semiquantitative analysis to assess the tissue injury index in examined sections. The results were expressed as the sum of individual score grades (0: no findings, 1: mild, 2: moderate, or 3: severe) for each of the following parameters: degeneration, cellular swelling, cellular vacuolization, necrosis, congestion, and hemorrhage (Survarna *et al*., 2013).

Statistical Analysis

We used SPSS (Statistical Package for Social Sciences) software (version 20) to perform the statistical analysis. All values were expressed as the mean and the standard error of mean (SEM). The means of different groups were compared using the one-way analysis of variance (ANOVA), followed by Tukey's post-hoc comparison tests. A p value <0.05 was considered statistically significant.

RESULTS

Effect on blood picture

Hematological results in the present study are illustrated in Table 1. The effect of oral administration of azithromycin (6 mg / kg bwt) for 10 successive days in male rats resulted in a significant decrease in RBCs, Hb, PCV%, WBCs, Platelets in a comparison with the control group. Concurrent administration of vitamin E (100 mg/kg bwt) orally with azithromycin for 10 successive days in male rats displayed a significant increase in RBCs, Hb, PCV%, WBCs, platelets when compared with azithromycin treated group. Simultaneous administration of sodium selenite (0.3 mg/kg bwt) orally with azithromycin for 10 successive days in male rats evoked a significant increase in RBCs, Hb, PCV%, WBCs, Platelets when compared with azithromycin group.

Effect on liver functions enzymes

The effect on liver enzymes activities (ALT, AST, ALP, and GGT) in the present study is illustrated in Table 2. Oral administration of azithromycin significantly increased ALT, AST, ALP, and GGT activities compared with the control group. However, oral administration of vitamin E or sodium selenite significantly reduced the elevated activities of ALT, AST, ALP, and GGT induced by azithromycin.

Effect on protein profile

The effect of oral administration of azithromycin alone or simultaneously with vitamin E or sodium selenite for 10 successive days on protein profile in male rats is represented in Table 3. The effect of oral administration of azithromycin in male rats elicited a significant decrease in total protein and albumin when compared with the control group. Oral administration of vitamin E or sodium selenite simultaneously with azithromycin in male rats revealed a significant increase in total protein and albumin when compared with azithromycin-treated group.

Effect on kidney function

The effect on kidney function (creatinine, urea, and uric acid) in the present study is demonstrated in Table 4. Oral administra-

Table 1. the effect of oral administration of azithromycin (6 mg /kg bwt), vitamin E (100 mg/kg bwt) and sodium selenite (0.3 mg/kg bwt) for 10 successive days on hematological picture.

Groups	Blood picture				
	RBCs ($\times 10^6$/µl)	Hb (g/dl)	PCV (%)	WBCs ($\times$ 103/µl)	Platelet ($\times 10^3$/µl)
Control (Saline)	7.65±0.05^{a}	12.57±0.37^{a}	39.90±1.05^{a}	8.70±0.43^{a}	673.30±13.50^{a}
Control (Olive oil)	7.48±0.2^{a}	13.27±0.87^{a}	39.50±1.03^{a}	8.17±0.80^{a}	669.30±20.30^{a}
Azithromycin	5.17±0.04^{d}	10.80±0.33^{b}	35.90±0.35^{b}	6.00±0.35^{b}	458.30±20.85^{c}
Vit E+ Azithromycin	6.05±0.14^{c}	12.80±0.53^{a}	37.80±0.1^{ab}	6.37±0.22^{a}	600.30±22.40^{bc}
SE+ Azithromycin	6.60±0.1^{b}	13.70±0.22^{a}	35.80±1.3^{b}	7.80±0.53^{a}	632.00±56.02^{ab}

Means within the same column having different alphabetical superscript litters are significantly different at $p<0.05$

Table 2. Effect of oral administration of vitamin E (100 mg/kg bwt) and sodium selenite (0.3 mg / kg bwt) on the liver enzymes activities of azithromycin (6mg / kg bwt) treated male rats for 10 successive days.

Groups	Liver enzymes activities			
	ALT (U/L)	AST (U/L)	ALP (IU/L)	GGT (IU/L)
Control (Saline)	23.0±3.2^{c}	28.0±1.7^{c}	101.3±1.85^{c}	19.0±3.7^{c}
Control (Olive oil)	19.6±2.7^{c}	32.7±1.4^{c}	102.3±4.6^{c}	24.3±4.7^{c}
Azithromycin	55.3±3.1^{a}	84.7±3.2^{a}	321.3±12.1^{a}	73.3±2.02^{a}
Vit E+ Azithromycin	38.0±1.5^{b}	50.3±0.9^{b}	204.6±3.2^{b}	44.3±1.5^{b}
SE+ Azithromycin	39.0±1.5^{b}	48.0±2.3^{b}	193.0±7.8^{b}	46 .3±3.1^{b}

Means within the same column having different alphabetical superscript litters are significantly different at $p<0.05$

Table 3. The effect of oral administration of azithromycin (6 mg /kg.b.wt), vitamin E (100 mg/kg bwt) and sodium selenite (0.3 mg / kg bwt) for 10 successive days on protein profile in male rats.

Groups	Protein profile		
	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control (Saline)	7.6±0.3^{a}	4.8±0.18^{a}	2.8±0.13^{a}
Control (olive oil)	7.3±0.35^{a}	4.9±0.09^{a}	2.7±0.5^{a}
Azithromycin	4.13±0.43^{c}	2.00±0.06^{c}	2.1±0.5^{a}
Vit E+ Azithromycin	5.6±0.23^{b}	3.67±0.14^{b}	1.9±0.33^{a}
SE+ Azithromycin	5.9±0.48^{b}	3.69±0.29^{b}	2.2±0.2^{a}

Means within the same column having different alphabetical superscript litters are significantly different at $p<0.05$

Table 4. The effect of oral administration of azithromycin (6mg /kg bwt), vitamin E (100 mg/kg bwt) and sodium selenite (0.3 mg / kg bwt) for 10 successive days on kidney functions in rats.

Groups	Parameters		
	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Control (Saline)	0.84±0.05^{c}	31.66±2.030^{c}	3.70±0.10^{c}
Control (olive oil)	0.73±0.06^{c}	36.30±1.80^{c}	3.70±0.26^{c}
Azithromycin	2.63±0.27^{a}	87.00±3.60^{a}	6.87±0.20^{a}
Vit E+ Azithromycin	1.40±0.05^{b}	48.66±0.88^{b}	5.04±0.33^{b}
SE+ Azithromycin	1.38±0.2^{b}	48.66±2.20^{b}	4.87±0.12^{b}

Means within the same column having different alphabetical superscript litters are significantly different at $p<0.05$

tion of azithromycin in male rats for 10 successive days showed a significant increase in creatinine, urea, and uric acid in a comparison with the control group. Meanwhile, oral administration of vitamin E or sodium selenite significantly decreased creatinine, urea, and uric acid levels in azithromycin-treated rats.

Antioxidant/oxidant status

The effect of oral administration of azithromycin alone or concurrently with vitamin E or sodium selenite on activities of GPX, SOD, and CAT, and malondialdehyde concentration (MDA) in male rats is represented in Table 5. Oral administration of azithromycin in male rats for 10 successive days produced a significant decrease in GPX, SOD, CAT, and GSH with a significant increase in MDA level in a comparison with the control group. However, concurrent administration of vitamin E or sodium selenite with azithromycin evoked a significant elevation in the activities of these enzymes with a significant reduction in MDA concentration in a comparison with azithromycin-treated rats.

Histopathological Findings

Group (1) (control)

Liver sections from control group showed preserved hepatic cords, portal triad's structures, vascular tributaries, biliary system, central veins, sinusoids, Von kupffer's cells and supporting stroma (Fig 1A, B). Kidney sections pointed out apparently normal nephron unites regarding the glomerular structures (tufts and Bowman's capsule) proximal and distal tubules besides loops of Henle. The collecting tubules, papillae, calyces, pelvis and vascular structures was normal. Neither inflammatory, nor degenerative or apoptotic changes were recorded in any of the examined parts (Fig. 1 C, D).

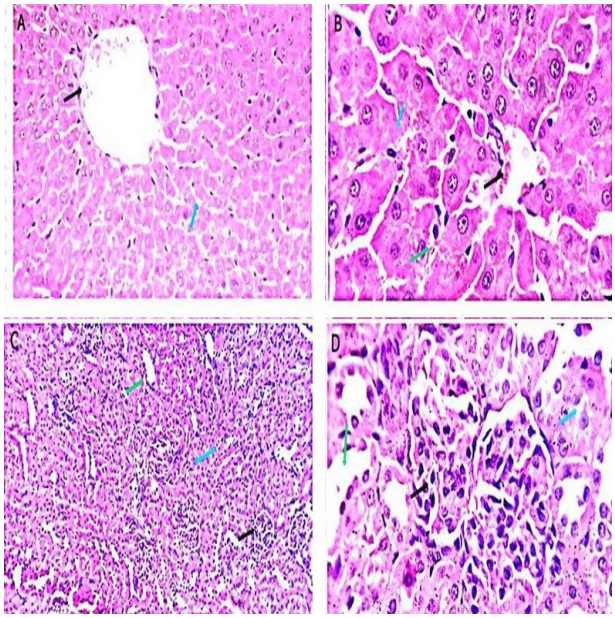

Fig. 1. Photomicrograph from rat's liver (A, B) of control negative group, showing preserved hepatic cords (blue arrows) portal triad's structures, vascular tributaries, central veins (blue-black) sinusoids and Von Kupffer's cells (green). Renal tissue (C, D) showing apparently normal nephron units regarding the glomerular structures (tufts and Bowman's capsule) (black arrows), proximal and distal collecting tubules (blue and green arrows) H&E X 100, 400.

Group (2) (olive oil)

Liver sections from olive oil-treated group showed preserved hepatic cords, portal triad's structures, vascular tributaries, biliary system, central veins, sinusoids, Von kupffer's cells and supporting stroma. Minute fat droplets were recorded in a very few hepatocytes (Fig. 2A, B). Kidney sections pointed out apparently normal nephron unites. The collecting tubules, papillae, calyces, pelvis and vascular structures was normal. No inflammatory changes were recorded in this group (Fig. 2 C, D).

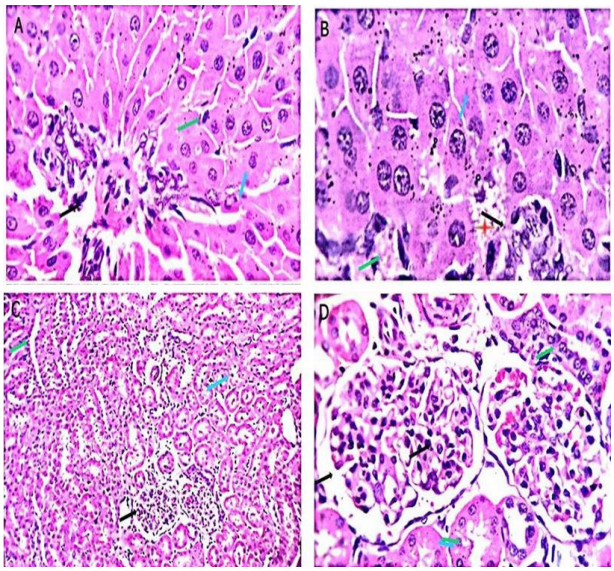

Fig. 2. Photomicrograph from rat's liver (A, B) of control group exposed to olive olive, showing preserved hepatic cords, (blue arrows), portal triad's structures, vascular tributaries, central veins (blue-black) sinusoids and Von Kupffer's cells (green arrows). Minute hepatocellular fat droplets are seen (red star). Renal tissue (C, D) showing apparently normal nephron units regarding the glomerular structures (tufts and Bowman's capsule) (black arrows), proximal and distal collecting tubules (blue and green arrows) H&E X 100, 400.

Group (3) (Azithromycin)

The examined serial sections from liver of this group delineated a characteristic hepatotoxic histopathological change represented by marked portal, perivascular and interstitial lym

Table 5. The effect of oral administration of azithromycin (6 mg /kg bwt), vitamin E (100 mg/kg bwt) and sodium selenite (0.3 mg / kg bwt) for 10 successive days on antioxidant oxidant status in male rats.

Groups	Antioxidant oxidant status				
	GPx (U/mL)	SOD (U/mL)	CAT (U/mL)	GSH (µmol/L)	MDA (µmol/L)
Control (Saline)	20.67±3.80^{a}	16.00±2.08^{ab}	5.50±0.5^{a}	29.00±1.70^{a}	7.90±0.18^{c}
Control (Olive oil)	22.30±1.20^{a}	19.00±1.50^{a}	5.20±0.5^{a}	30.70±1.50^{a}	8.00±0.28^{c}
Azithromycin	9.70±0.88^{b}	7.33±0.90^{c}	1.00±0.1^{c}	14.33±1.45^{c}	28.70±0.88^{a}
Vit E+ Azithromycin	18.70±0.88^{a}	11.30±2.50^{bc}	3.30±0.3^{b}	21.00±1.15^{b}	16.00±2.60^{b}
SE+ Azithromycin	20.30±1.20^{a}	16.00±2.08^{ab}	3.60±0.3^{b}	22.30±0.33^{b}	19.60±1.20^{b}

Means within the same column having different alphabetical superscript litters are significantly different at $p<0.05$

pho-plasmacytic infiltration with partial replacement of the hepatic parenchyma. Focal necrotic areas replaced by macrophages and a few lymphocytes were also seen. The biliary epithelium was remarkably proliferated. Von Kupffeer cells were hypertrophied and or hyperplastic. An increase in the number of circulating sinusoidal lymphocytes was also seen (Fig. 3 A, B).

Kidney sections of azithromycin-treated group showed highly dilated renal blood vessels, lymphangiectasia, caliectasis, collecting tubular dilatation, some were impacted by homogenous eosinophilic structures materials, and others showed hyaline casts. Perivascular round cells infiltration, edema, tubular epithelial degenerative changes, glomerular lobulation and or atrophy and mild peri -papillary fibrosis were recorded (Fig. 3 C, D).

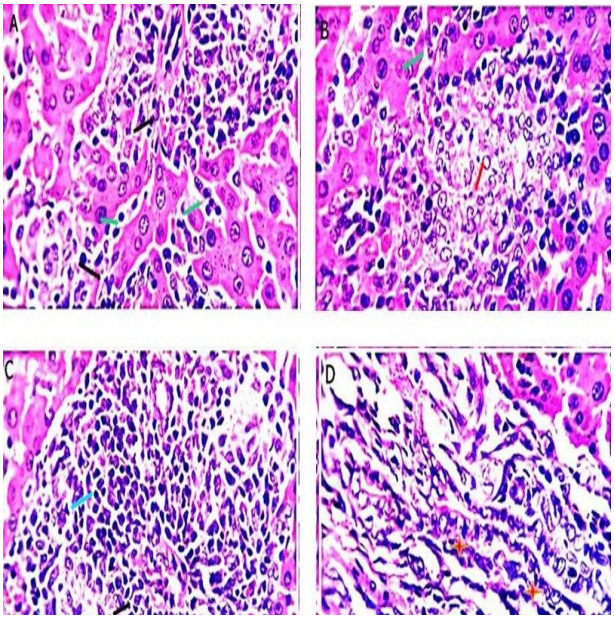

Fig. 3. Photomicrograph from rat's liver (A, B) of Azithromycin treated group, showing marked portal, perivascular, and interstitial lymphoplasmacytic infiltration with partial replacement of the hepatic parenchyma (black and blue arrows). Focal necrotic areas are replaced by macrophages and a few lymphocytes are seen (red arrow). The biliary epithelium was remarkably proliferated (red stars). Von Kupffer cells were hypertrophied and or hyperplastic (green arrow). H&E X 400. Nephrotoxic changes were represented by highly dilated renal blood vessels, lymphangiectasia, caliectasis, and collecting tubular dilatation, some were impacted by homogenous esinophilic structure materials, and others showed hyaline casts. Perivascular round cell infiltration, edema, tubular epithelial degenerative changes, glomerular lobulation and or atrophy and mild peri -papillary fibrosis were recorded (C, D).

Group (4) (Azithromycin +Vitamin E)

The examined serial sections from liver of this group declared a mild to moderate ameliorative effect of vitamin E. Most of the hepatic parenchyma including hepatic cords and sinusoids were apparently normal, however some cases (35-40%) revealed mild to moderate hepato-portal vascular congestion and biliary proliferation together with sporadic hepatocellular degenerative and apoptotic changes (Fig. 4 A, B). Kidney sections from this group revealed mild histopathological alternations and seemed to be normal (Fig. 4 C, D).

Group (5) (Azithromycin + Selenium)

The examined serial sections from liver of this group revealed a moderate ameliorative effect of selenium. Most of the hepatic parenchyma including hepatic cords and sinusoids were apparently normal. Some cases (15-20%) revealed mild hepato-portal vascular congestion and biliary proliferation together with sporadic hepatocellular degenerative and apoptotic changes. Mild portal lymphocytic infiltration was seen (Fig. 5 A, B). Histopathological finding of kidney of this group was apparently normal (Fig. 5 C, D).

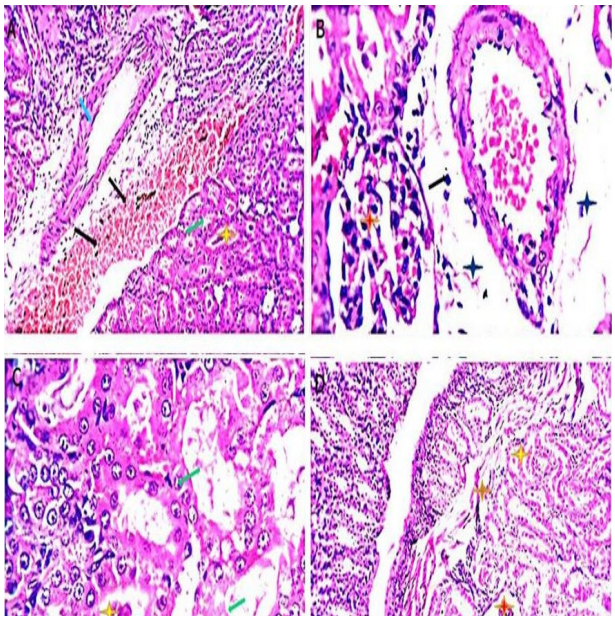

Fig. 4. Photomicrograph from rat's liver (A, B) of Azithromycin-Vit.E treated group, showing mild to moderate hepato-portal vascular congestion (back arrows) and biliary proliferation (red stars) together with sporadic hepatocellular degenerative and apoptotic change(black star). H&E X 200, 400. Renal sections of this group pointed a sustained nephrotoxic changes with a milder degree as compared with the Azithromycin treated group. Still moderate renal vascular dilatation, perivascular edema, collecting tubular cystic changes with parial impaction of homogenous red esinophilic materials, focal renal epithelial degeneration , intra-tubular hyaline casts formation and mild glomerular lobulation could be recorded in some but not all cases (35-40%) (C, D).

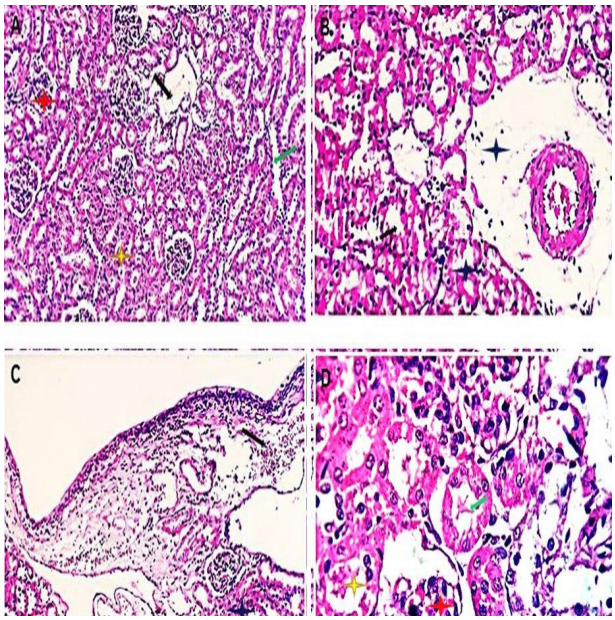

Fig. 5. Photomicrograph of rat exposed to Azithromycin-selenium treated group. Rat's liver of this group revealed a moderate ameliorative effect of selenium. Most of the hepatic parenchyma including hepatic cords and sinusoids were apparently normal (A, B), kidney (C, D) of the Azithromycin-Selenium-treated group, showing mild renal vascular dilatation (black arrows), perivascular edema (black stars), intra-tubular hyaline casts formation (yellow stars) and mild glomerular lobulation (red stars). H&E X 100, 200, 400.

DISCUSSION

The macrolide family of antibiotics includes azithromycin. According to Bari *et al.* (2022) it is useful against bacterial and inflammatory diseases, such as sore throats. It displayed a range

of effects on several well-known viruses, including influenza, enteroviruses, rhinoviruses, and the severe acute respiratory syndrome coronavirus (SARS-CoV). Due to its antiviral and immunomodulatory properties, azithromycin has garnered interest as a promising COVID-19 therapy option (Khoshnood *et al*., 2022). The results of the current investigation showed that oral azithromycin administration in male rats for 10 consecutive days resulted in a significantly lower level of GPX, SOD, CAT, and GSH and a significantly higher level of MDA when compared to the control group. Reactive oxygen species (ROS), also known as oxygen free radicals or other substances of a similar nature, can be used to detect the presence of oxidative stress. Antioxidants and damaged versions of vital biomolecules, such as MDA produced because of lipid peroxidation, can also be used to detect the presence of oxidative stress. One or more antioxidants may become depleted as a result of increased ROS production in vivo. According to Haliwell and Gutteridge (1998), loss of specific antioxidants, such as ascorbate or glutathione, can be used as a gauge of oxidative stress. The reduced antioxidant enzymes and the increased MDA are strong indicators that azithromycin has the potential to cause oxidative stress. This earlier result is consistent with Olayinka and Ore's findings from 2014, which indicated that rats treated with azithromycin displayed a substantial decrease in SOD and catalase activity when compared to the control group. Similar to this, Mansour *et al*. (2021) found azithromycin given at dose 30 mg/kg per os daily for two weeks in male albino rats led to a large rise in MDA concentration as well as a significant fall in GSH concentration in heart tissue compared to control. Similar to this, Omara *et al*. (2021) reported that rats treated with azithromycin had significantly higher serum levels of GSH, glutathione peroxidase (GPx), and MDA. with lower ALT, AST, ALP, and GGT activities, and significant histopathological changes. According to Cantin and Woods (1993); Mingeot-Leclerq and Tukens (1999) and Rybak and Whitworth (2005), free radicals are a significant factor in the harm that drugs cause to the liver, kidneys, and other organs. The etiology of the oxidative stress linked to diabetes mellitus, hypertension, and aminoglycoside nephrotoxicity is due to their increased vascular tone and tubuloglomerular degeneration of the kidney (Schnackenberg, 2002). According to Olayinka and Ore (2014), azithromycin given in rats resulted in a significantly higher level of ALP, ALT, AST, and GGT when compared to control animals. These findings are in line with their findings. The liver tissues also experienced modest per portal cellular infiltration, portal congestion, and protein casts in the tubular lumen, cortical congestion, and hemorrhage. Similar to this, Sakurai (2018) reported that azithromycin caused minor vacuolation in the renal tubules of the inner stripe of the outer medulla, cortex, glomerulus, and transitional epithelium of the kidney, resulting in phospholipidosis in rat kidney. Azithromycin therapy in humans at a dose of 500 mg IV resulted in a considerable increase in serum ALT and AST while ALP remained normal, according to Ellison and Blackwell (2021). Similar to this, Al- Kaissy (2011) reported that mice given azithromycin at a dose of 250 mg/kg/day for three days showed a significant increase in GPT and AST activation and a decrease in total protein compared to the control group.

Similar to the recorded histopathological changes in the current study, a number of histological changes were also discovered in the liver tissues by Omara *et al*. (2021), including hydropic degenerative and edematous portal veins, periductular fibrosis with lymphocytes, and coagulative necrosis of hepatic cells. Our results supported Caroly and Sarahv's (2021) conclusion that azithromycin increased ALT and AST levels. Similarly, Olayinka and Ore (2014) found that rats exposed to azithromycin developed liver and kidney damage. In the liver and renal histological tissues sections, there was substantial tissue damage.

In the present work, vitamin E or selenium administrations significantly decreased ALT, AST, ALP, and GGT activities, creatinine, urea, and uric acid levels and MDA concentration in azithromycin-treated rats. Moreover, both vitamin E and selenium significantly increased hematological parameters, protein profile (total protein, albumin, and globulin) and antioxidant enzyme activities (GPX, SOD, GSH, CAT) in rats treated with azithromycin with a significant improvement in histopathological pictures of hepatic and renal tissues. The antioxidant and free radical scavenging activities of both vitamin E and selenium may be the cause behind the ameliorative effects for azithromycin-induced tissue toxicity. In agreement with this assumption, Diverse action mechanisms are displayed by selenium. Selenoproteins, which play a significant role in many of the diverse functions of selenium, are formed when selenium is integrated into a variety of proteins. By serving as a cofactor for glutathione peroxidase through cellular metabolism, selenium lowers oxidative damage (Tinggi, 2008). Cell membranes and organelles are protected from oxidative damage by selenium and vitamin E collectively. Selenium can thereby boost the host's defiance systems and immunological system. Selenium's significance in the endocrine system is established by its role in the synthesis of active thyroid hormone. The mineral interacts with iodothyronine deiodinase, an enzyme that changes inactive thyroid hormone (T4) into active thyroid hormone (T3) (Shreenath *et al*., 2018).

CONCLUSION

Vitamin E at a prophylaxis dose (100 mg/kg bwt) and selenium at 0.3 mg/kg bwt may exert beneficial effects for the protection of liver and kidney against azithromycin-induced damage.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this research paper.

REFERENCES

Al- Kaissy, A.M., 2011. Biochemical studies on the effect of Toxoplasma infection on liver and kidney functions in mice. Journal of Al-Ma'moon College 18, 120-127.

Amara, I.B., Soudani, N., Hakim, A., Troudi, A., Zeghal, K.M., Boudawara, T., Zeghal, N., 2011. Selenium and vitamin E, natural antioxidants, protect rat cerebral cortex against dimethoate-induced neurotoxicity Pest. Biochem. Physiol. 101, 165–174.

Atef, M., Al-Attar, E., 2011. Antioxidant effect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi. J. Biol. Sci. 18, 63–72.

Bari, S.B., Adhikari, S.R., Bambodkar, S.S., Bhilare, S.G., Bharati, D.K., 2022. Observational study of effects of azithromycin and nutraceuticals on COVID-19 patients. World J. Pharm. Res. 11, 2377-2395.

Buttarello, M., Plebani, M., 2008. Automated blood cell counts: State of the Art, Amer. J. Clin. Pathol. 130, 104–116.

Cantin, A., Woods, D.E., 1993. Protection by antibiotics against Myeloperoxidase-dependent cytotoxicity to lung epithelial cells in vitro. J. Clin. Invest. 91, 38-45.

Coulombe, J.J., Favreau, L., 1963. A new simple semimicro method for colorimetric determination of urea. Clin. Chem., 9, 102-108.

Draperi, H.H., Hadly, N., 1990. Malondialdehyde determination as an index of lipid peroxidation. Method Enzymol. 186, 421-431.

Ellison, C.A., Blackwell, S.B., 2021. Acute hepatocellular injury associated with azithromycin. J. Pharm. Prac. 34, 493-496.

Goldman, R.C., Fesik, S.W., Doran, C.C., 1990. Role of protonated and neutral forms of macrolides in binding to ribosomes from gram-positive and gram-negative bacteria. Antimicrob. Agents Chemother. 34, 426-31.

Haliwell, B., Gutteridge, J.M.C., 1998. Free Radicals in Biology and Medicine. Haliwell B. Gutteridge, JMC Eds. 351-429.

Inoue, Y., Inoue, M., Saito, M., Yoshikawa, H., Tamiya, E., 2017. Sensitive detection of glycated albumin in human serum albumin using electrochemiluminescence. Anal. Chem. 89, 5909-5915.

Kalender, S., Kalender, Y., Ates, A., Yel, M., Olcay, E., Candan, S., 2002. Protective role of antioxidant vitamin E and catechin on idarubicin-induced cardiotoxicity in rats. Braz. J. Med. Biol. Res. 35, 1379-1387.

Khoshnood, S., Shirani, M., Dalir, A., Moradi, M., Haddadi, M.H., Sadeghifard, N., Heidary, M., 2022. Antiviral effects of azithromycin: A narrative review. Biomed. Pharmacother. 147, 112682.

Kind, P.R.N., King, E., 1954. Estimation of plasma phosphatase by deter-

mination of hydrolysed phenol with amino-antipyrine. J. Clin. Pathol. 7, 322.

Mansour, B.S., Salem, N.A., Kader, G.A., Abdel-Alrahman, G., Mahmoud, O.M., 2021. Protective effect of Rosuvastatin on Azithromycin induced cardiotoxicity in a rat model. Life Sci. 269, 119099.

Mingeot-Leclercq, M.P., Tulkens, P.M., 1999. Aminoglycosides: nephrotoxicity. Antimicrob. Agents Chemother. 43, 1003-1012.

Mukai, K., Tokunaga, A., Itoh, S., Kanesaki, Y., Ohara, K., Nagaoka, S., Abe, K., 2007. Structure-activity relationship of the free-radical-scavenging reaction by vitamin E (alpha-, beta-, gamma-, delta-tocopherols) and ubiquinol-10: pH dependence of the reaction rates. J. Phys. Chem. B Condens. Matter Mater Surf Interfaces Biophys. 111, 652–662.

Olayinka, E.T., Ore, A., 2014. Influence of Azithromycin treatment on hepatic lipid peroxidation and antioxidant defence systems of rats. Brit. J. Pharma. Res. 4, 240-256.

Olsvik, P.A., Berntssen, M.H.G., Søfteland, L., 2015. Modifying effects of Vitamin E on chlorpyrifos toxicity in Atlantic salmon. PLOS ONE. 10, e0119250.

Omara, F., Aziz, S. A., El-Sheikh, S. M., Said, M.A.A., 2021. Ascorbic acid attenuated the hepatic parenchymal necrosis induced by azithromycin-etoricoxib interaction in rats. J. Anim. Health Prod. 9, 42-48.

Phillips, R.W., Booth, N.H., Mc Donald, L.E., 1982. Fat soluble vitamins, In: Jones Veterinary Pharmacology and Therapeutics, Kalyani Publishers, New Delhi. pp.1134.

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271-278.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Amer. J. Clin. Pathol. 28, 56-63.

Rybak, L.P., Whitworth, C.A., 2005. Ototoxicity: therapeutic opportunities. Drug Dis. Today 10, 1313-21.

Sakurai, T., Kamio, K., Sasaki, K., Nishimoto, T., Yamaguchi, J.I., Sasaki, M., Tsutsumi, S., 2018. Imaging mass microscopy of kidneys from azithromycin-treated rats with phospholipidosis. Amer. J. Pathol. 188, 1993-2003.

Schnackenberg, C.G., 2002. Oxygen radicals in cardiovascular renal disease. Curr. Opin. Pharmacol. 2, 121-125.

Shreenath, A.P., Ameer, M.A., Dooley, J., 2018. Selenium deficiency. https://europepmc.org/article/nbk/nbk482260.

Shreenath, A.P., Ameer, M.A., Dooley, J., 2022. Selenium Deficiency. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2022. PMID: 29489289.

Survarna, K., Lyton, C., Bancroft, J.D., 2013. Bancroft's theory and practice of histological Techniques. 7th Ed. Oxford, Churchil Livingston, Elsevier, England. pp.654

Tinggi, U., 2008. Selenium: its role as antioxidant in human health. Environ. Health Prev. Med. 13, 102-108.

Traber, M.G., Atkinson, J., 2007. Vitamin E, Antioxidant and Nothing More. Free Radic. Biol. Med. 43, 4–15.