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Protective effects of lycopene against Tilmicosin induced hepatic and pulmonary toxicity in male albino rats

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A B S T R A C T

The hereby study was designed to verify the deleterious effects of tilmicosin (TIL) on rats' hepatic and lung tissues. Lycopene (LYC) is an antioxidant phytochemical carotenoid. Sixty male albino rats were used throughout the experiment. They were divided into six groups (No = 10), as follows: Group, 1 (control 1), injected subcutaneous (s/c) with isotonic saline solution, Group, 2 (control 2) administrated 0.2 ml olive oil oral by stomach tube daily and kept also as control and scarified after 15 days. Group, 3 (LYC group) administrated 10 mg / kg of lycopene dissolved in olive oil and given by stomach tube daily and scarified after 15 days Group, 4 (TIL group) administrated 60 mg / kg of tilmicosin single s/c dose and scarified after 5 days. Group, 5 (Prophylactic group) administrated 10 mg / kg of lycopene for 15 days, then 60 mg / kg tilmicosin s/c and scarified after 5 days. Group, 6 (Treatment group) administrated 60 mg / kg tilmicosin once s/c and administrated lycopene for 10 days then, the half of the group scarified after 5 days from lycopene administration and the other half after 10 days. LYC administration reduced the cytotoxic effects of tilmicosin on hepatic tissue, through improving the liver function biomarkers as alkaline phosphates (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and oxidant-antioxidant state. Increased ALP, ALT and AST activity may indicate that tilmicosin at the tested doses caused significant changes in hepatic tissues. Our findings reveal that LYC credited to have a noticeable protective effect against tilmicosin oxidative injury of the liver and lung tissue.

Key words Antioxidant, Lycopene, Oxidative damage, Tilmicosin

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1. INTRODUCTION

Hepatitis remains a clinical challenge and a problem of great importance in the developing and underdeveloped world. The viral hepatitis, particularly, hepatitis B is the most common form of acute hepatitis. Acute hepatitis can have serious health effects including mortality. There is no specific treatment for acute hepatitis, therefore proper care is essential to maintain comfort and adequate nutritional balance (Abdulazeez and Thiruvengadam, 2013).

Lycopene is the most abundant carotenoid present in human plasma, accounting for approximately 50% of all plasma carotenoid content (Stahl et al., 1996). A number of studies showed that lycopene inhibited the growth of human cancer cells grown in cultures. The growth-inhibitory effects of

lycopene were observed not only in lung cancer cells, but also in other cell types, including prostate, breast, hepatoma, stomach, colon and oral cancer cells (Park et al., 2005; Palozzaet al., 2010). LYC credited to have a noticeable protective effect versus provoked oxidative injury and apoptosis of the liver tissue (Abdel-Rahman, 2018).

Tilmicosin was synthesized from tylosin for veterinary use only. It has a potent antimicrobial with broad-spectrum activity against the bacterial agents involved in the bovine respiratory disease complex (Lakritz et al., 2002). TIL is similar to other macrolides, this drug has a long half-life and maintains high concentrations both in lung and milk (DeRosa et al., 2000, Frank et al., 2000; Naccari et al., 2001; Fittipaldi et al., 2005; Zhang et al., 2016). TIL cause significant hepatotoxic effects demonstrated by the histopathological findings of the liver as severe hepatic cell necrosis and degenerative changes with proliferation of the Kupffer cells (Abo El-Ela and El Banna 2017). The aims of this study were to evaluate the protective effect of Lycopene on the changes of liver and lung induced by tilmicosin toxicity in male albino rats.

2. MATERIALS AND METHODS

2.1. Chemicals:

Tilmicosin phosphate injection, solution. Each 1mL contains 300 mg of tilmicosin, USP as tilmicosin phosphate in 25% propylene glycol, phosphoric acid as needed to adjust pH and water for injection.

Lycopene is a red crystalline substance (C 40 H 56) that is the main pigment of certain fruits, as the tomato and paprika, and is a precursor to carotene in plant biosynthesis . It was obtained from DEBEIKY Pharmatheutical Company.

2.2. Experimental design

Sixty male albino rats (western strain) were divided in to 6 groups each of 10 rats. Group I administrated 0.5 ml saline s/c and kept as control and scarified after 5 days. Group II administrated 0.2 ml olive oil orally by stomach tube daily and kept also as control and scarified after 15 days. Group III administrated therapeutic dose of lycopene (10 mg / kg) dissolved in olive oil and given by stomach tube daily and scarified after 15 days (Gajowik et al., 2017; Kaya et al., 2017). Group IV administrated 60 mg / kg of tilmicosin single dose s/c and scarified after 5 days. The therapeutic dose is 10mg/kg (Equivalent to 1ml / 30 kg) given as a single subcutaneous injection (Avci and Elmas, 2014; Said et al., 2016). Group V (Prophylactic group) administrated 10 mg / kg of lycopene for 15 days then 60 mg / kg tilmicosin s/c and scarified after 5 days and Group VI (Treatment group) administrated 60 mg / kg tilmicosin one dose s/c and administrated lycopene for 10 days then, the half of the group scarified after 5 days from lycopene administration and the other half after 10 days. All rats are kept under observation all over the experimental period.

2.3. Sampling:-

Blood samples were collected by puncture of retro orbital plexus from each rat in each group of experiment. It collected in clean dry centrifuge tubes, allowed to stand for one hour at room temperature till clotted and centrifuged at 3000rpm for fifteen minutes, for serum separation, and then kept in -20C for biochemical analysis.

Tissue specimens (lung and liver) were collected and fixed in 10% formol saline for histopathological studies.

2.4. Biochemical analysis:-

ALT and AST according to Klin et al. (1972), ALP was determined according to Berth and Delanghe (2004).

2.5. Histopathological examination:-

Autopsy samples were taken from the liver and lung of rats in different groups and fixed in 10% formol saline for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for

sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, and stained by hematoxylin & eosin stain for routine examination through the light electric microscope (Banchroft et al., 1996).

2.6. Statistical analysis:-

The data were analyzed for obtaining mean, standard deviation (SD) and statistical comparisons between means of different groups. The statistical analyses were done by one-way ANOVA and DUNCAN test using SPSS program version 11. P value < 0.05 was assumed for statistical significance.

3. RESULTS

3.1. *The biochemical parameters*

The biochemical parameters in the tilmicosin group in the present study were significantly different from those of control group. ALP, ALT and AST activity values at tested doses of tilmicosin were significantly (P<0.05) increased compared to those of the controls. Meanwhile, TIL +LYC (treatment group) and LYC+TIL (prophylactic group) exhibited rapid significant ($P < 0.05$) improvement in these parameters when compared to the TIL group but still significantly ($P < 0.05$) increased from the control one. Rats supplemented with LYC only did not differ from the control group (Table 1).

Table (1) Effects of tilmicosin and lycopene on serum levels of ALP, ALT and AST of adult male albino rats.

	ALT (U/L)	AST (U/L)	ALP (U/L)
Control saline	40±3.84	44.54±5.49	615.32±23.75
Control oil	52.48±2.31	62.38±2.01	743.97±43.67
Lycopene	36.42±3.72	56.18±3.82	399.48±39.54
Tilmicosin	92.12±5.64	127.12±19.65	885.35±92.13
Treatment for 5 days	61.02±2.52	84.24±2.26	266.14±48.81
Treatment for 10 days	56.32±1.93	65.66±3.14	284.68±32.38
Prophylactic	66.02±2.36	84.82±3.94	339.94±53.76

3.2. Histopathological examination of lungs:

There were no histopathological alterations of lungs in control groups and the normal histological structure of the bronchiole with the surrounding air alveoli and blood vessels in control saline group (Photo 1) and in control oil (Photo 2). Concerning to the histopathological changes of lungs in the lycopene treated rat, there were no histopathological alterations as recorded in (Photo 3).

Lungs of tilmicosin treated rats showed peribronchiolar lymphoid hyperplasia with congestion in the blood vessels (Photos 4 & 5).

The bronchiolar lining epithelium showed hyperplasia with polyps formation (Photo 6). While in lungs of treated group with tilmicosin then lycopene for 5 days: There was congestion in the peribronchiolar blood vessels (Photo 7). While in treated group for with lycopene 10 days: The bronchiolar epithelial cells showed hyperplasia with polyps formation (Photo 8). Lungs of prophylactic group showed no histopathological alterations as recorded in (Photo 9).

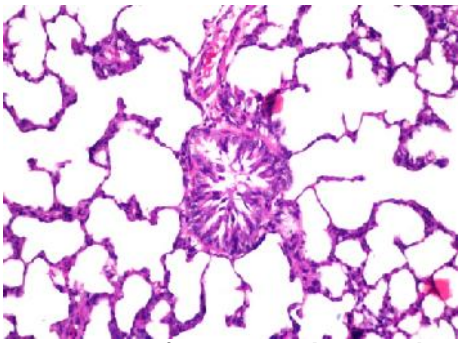

Photo (1): Lung of rat in control groups showing normal histological structure.

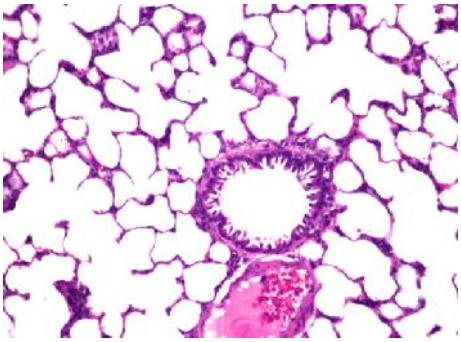

Photo (2): Lung of rat in control groups showing normal histological structure.

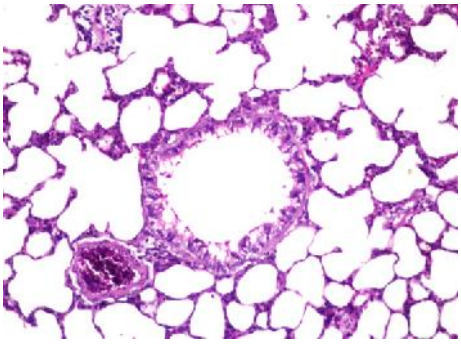

Photo (3): Lung of lycopene group showing normal histological structure

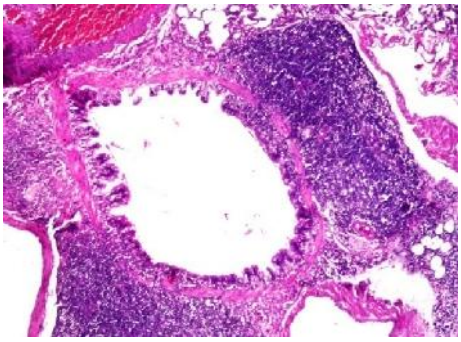

Photo (4): Lung of tilmicosin group showing peribronchiolar lymphoid hyperplasia with congestion of blood vessels.

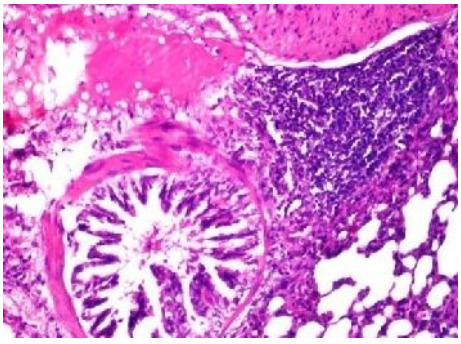

Photo (5): Lung of tilmicosin group showing higher magnification of photo (4) to define the peribronchiolar lymphoid hyperplasia.

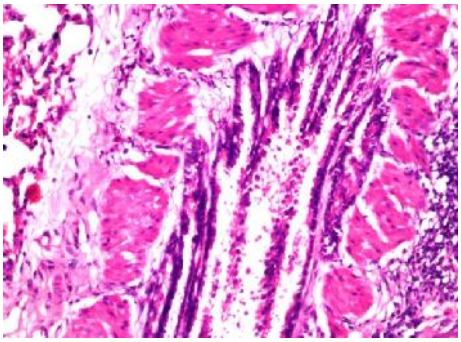

Photo (6): Lung of tilmicosin group showing hyperplasia in the lining bronchiolar epithelium with polyps formation.

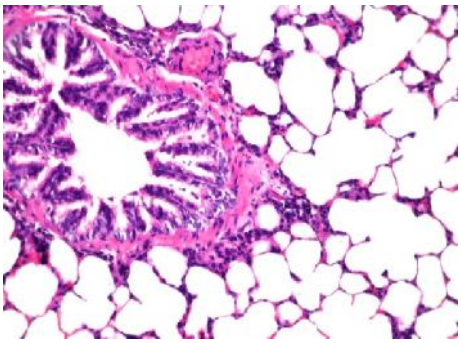

Photo (7): Lung of treated group for 5 days congestion in peribronchiolar blood vessels.

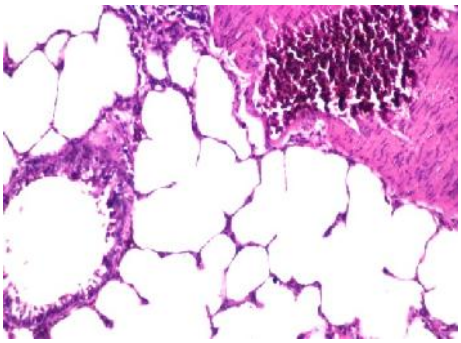

Photo (8): Lung of treated group for 10 days showing hyperplasia with polyps formation.

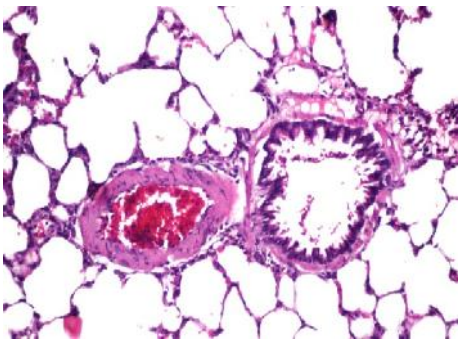

Photo (9): Lung of rat in prophylactic groups showing normal histological structure.

3.3. Histopathological examination of liver:
There were no histopathological alterations of Liver in control saline and control oil groups and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma were recorded in (Photos 10 & 11). Concerning to the histopathological changes of liver in the lycopene treated rat, there were no histopathological alterations as recorded in (Photo 12).
Dilatation and congestion were detected in tilmicosin treated group in both central and portal veins associated with degeneration in the hepatocytes in the surrounding area (Photo 13), while the portal area showed edema (Photo 14).
Liver of treated group with tilmicosin then lycopene for 5 days: Diffuse Kupffer cells proliferation was detected in between the degenerated hepatocytes (Photo 15). The portal area showed congestion in the portal vein with few inflammatory cells infiltration (Photo 16). While in treated group for 10 days: The portal showed thickening by edema associated with mild congestion in the portal vein (Photo 17). Liver of prophylactic group showed no histopathological alteration as recorded in (Photo 18).

4. DISCUSION

The macrolide antibiotic tilmicosin is being used in treatment of respiratory diseases in different animal species including cattle, swine (Moran et al., 1997), rabbit (McKay et al., 1996) and rat (Modric et al., 1999).

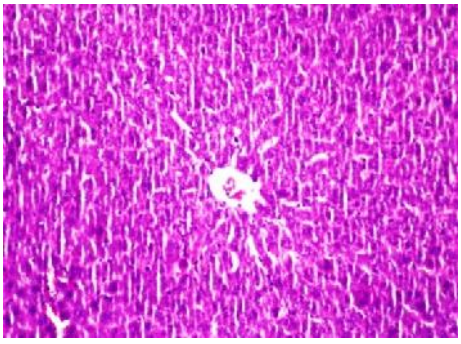

Photo (10): Liver of rat in control groups showing normal histological structure of central vein and surrounding hepatocytes in the parenchyma.

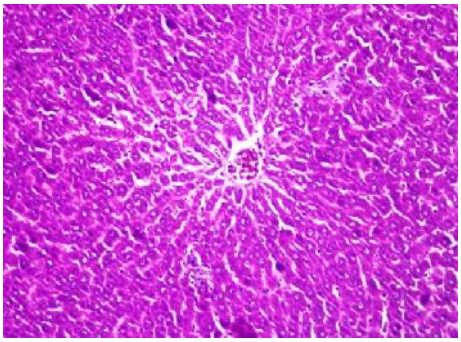

Photo (11): Liver of rat in control groups showing normal histological structure of central vein and surrounding hepatocytes in the parenchyma.

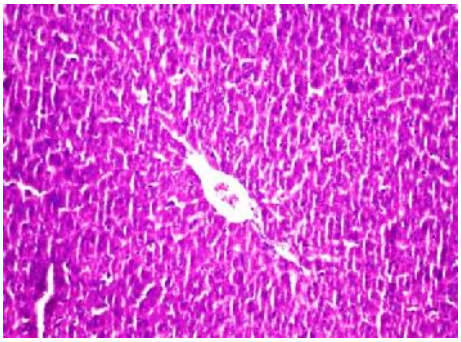

Photo (12): liver of lycopene group showing normal histological structure

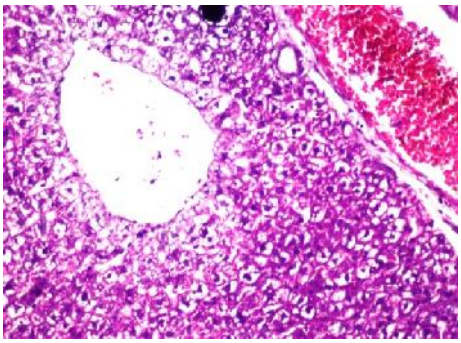

Photo (13): Liver of tilmicosin group showing dilatation of central and portal veins with degeneration in the hepatocytes in surrounding areas.

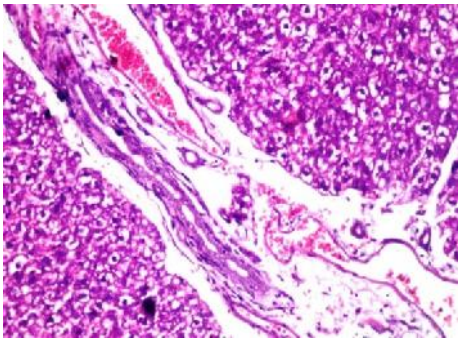

Photo (14): liver of tilmicosin group showing edema in portal area with degeneration in the hepatocytes in surrounding hepatocytes.

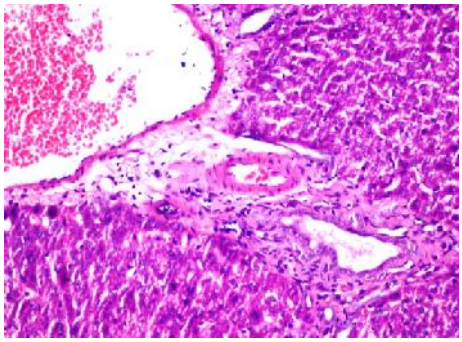

Photo (15): liver of treated group for 5 days showing diffuse Kupffer cells proliferation in between the degenerated hepatocytes.

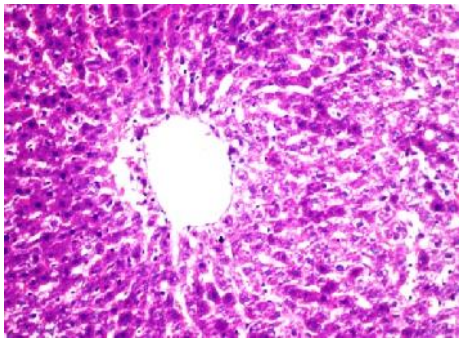

Photo (16): Liver of treated group for 5 days showing congestion in the portal vein with edema and inflammatory cells infiltration in portal area.

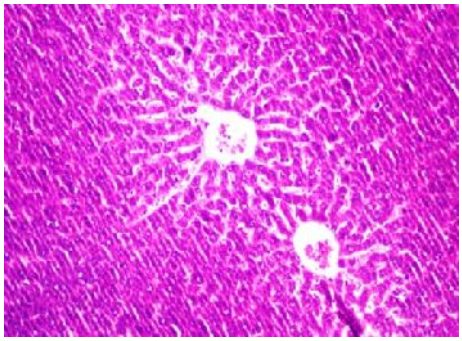

Photo (17): Liver of treated group for 10 days showing thickening in the portal area by edema with mild congestion in portal vein.

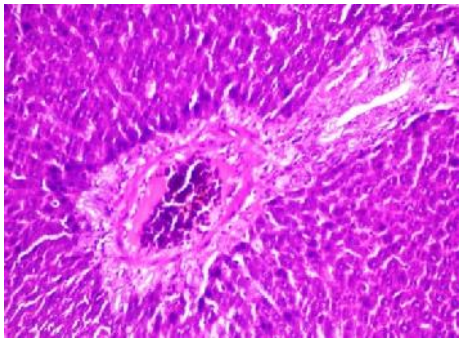

Photo (18): liver of prophylactic group showing normal histological structure.

Therapy with macrolides is sometimes associated with gastrointestinal disturbances, jaundice, and transient swelling at injection site (Barragry & Powers, 1994). Macrolides are metabolized by the liver and achieved a high concentration in it. Therefore, tilmicosin might generally hepatotoxic (Barragry, 1994) and cause changes in ALP, ALT and AST concentrations (Yazar, 2004). ALT is a cytoplasmic enzyme and its increased level in plasma is an indication of mild injuries caused by the drug to the liver. While AST is a mitochondrial enzyme whose increased activity in plasma reflects severe hepatic tissue injury (Crook, 2006). It should be noted that although ALP is formed mostly in the liver, yet, it is nonspecific to hepatic injury as it is formed by other tissues as bone, kidney and placenta. Results of the present data indicate that tilmicosin, particularly at high dose level, may be hepatotoxic (Gheith et., al., 2015).

Nevertheless, its increase along with AST and ALT may refer to that its source of elevation is hepatic. Degenerative changes in the liver tissue showed in photo (13&14). These lesions may have occurred as a result of metabolism of the drug by the liver which serves as the primary organ of biotransformation (Abo El-Ela and El Banna, 2017). Also, this attributed to the hepatic necrosis, dilatation in the central vein and severe vascular inflammation caused by macrolides antibiotic groups, this come in accordance with (King-Wing et al., 2014) as reported that macrolides inhibit the P-glycoprotein transport of the liver which responsible for ATP transfers.

Lung concentrations of tilmicosin remain above the MIC of M. haemolytica (3.15 lg/mL) for at least 72 h following a single SC injection at a dose of 10 mg/kg (DeRosa et al., 2000) thus considered the cause of histological pulmonary changes in lung of rats administrated tilmicosin alone. Prolonged period of tilmicosin concentration in lungs considered the main cause histopathological changes as these changes increased in treatment group for 10 days than that in treatment for 5 days (Photo 7 & 8).

Lycopene might react with peroxy radicals, which are formed in propagation phase of lipid peroxidation to form carbon-centered radical. The carbon centered radical reacts readily and reversibly with oxygen to form a new chain carrying peroxyl radicals, which are highly stable forms than ROS and thereby inhibiting lipid peroxidation (Aggarwal et al., 2009). The free radical scavenging effect of lycopene may be attributed by its potent antioxidant property (Srinivasan et al., 2007). Reducing apoptosis and thus keeping hepatic integrity and prevented the liberation of hepatic enzymes into the blood of rats (Abdel-Rahman, 2018).

5. CONCULOSIONS

The current study demonstrated the protective effects of LYC against TIL hepatic oxidative injury. Antioxidant effects of LYC led to cleared tilmicosin metabolites rapidly and decreasing the exposure of hepatic cells to their harmful effects. All these effects keep hepatic integrity and prevented the liberation of hepatic enzymes into the blood of rats. lycopene effectively combated oxidative damage and protected antioxidant defense status of the cell. Pretreatment of lycopene also offers protection against cell damage and confirms the antioxidant nature of the phyto-nutrient against experimental hepatitis.

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