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Hepatoprotective Effect of Luteolin against Azithromycin Induced Hepatotoxicity

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Abstract:
Azithromycin, a macrolide, is broad spectrum antibacterial in numerous countries; however, it has severe side effects such as hepatotoxicity. The current study was designed to determine the hepatoprotective effect of Luteolin (50 mg/kg body weight) against azithromycin (30mg/kg body weight) induced hepatotoxicity. Lut treatment showed improvement of the hepatic damage induced by AZ via ameliorating the elevation ofthe liver enzyme activities such as alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma-glutamyl transferase [GGT]. Lut also restore the balance of antioxidant defense and oxidative stress by increasing the activities of catalase (CAT) and superoxide dismutase (SOD) and reduced glutathione (GSH) levels as well as by reducemalondialdehyde levels. Moreover, Lut showed anti-inflammatory effect by upregulation of interleukin IL-10 with downregulation of downregulation of IL-6. It can be concluded that Luteolinhas hepatoprotective agent against hepatotoxicity caused by Azithromycin administration.
Keywords*:* Azithromycin; Hepatoprotective; Oxidative stress; Luteolin

Introduction

Azithromycin (AZ) is as semisynthetic antibiotic linked to macrolide family which represents a 2nd generation of macrolide modifications(Abeer, 2015). AZ is widely used against both types of bacteria and other pathogens; AZ prohibits bacterial growing by intervening with their protein synthesis through inhibiting mRNA translation(El-Shitany and El-Desoky, 2016). According to previous literatures, prolonged use of AZ or with high dose was concomitant with severe side effects such as hepatotoxicity, nephrotoxicity, cardiotoxicity and other tissues damage(Varano et al., 2017, Al-Darraji et al., 2018). Strong evidence documented that AZ induced hepatotoxicity in rats(Singh et al., 2016). Hepatotoxic mechanism of AZ is not clearly understood, but several studies proposed some mechanisms involved in hepatotoxicity such as overproduction of reactive oxygen species (ROS), mitochondrial dysfunction and inflammation(Paulose et al., 2016). Over generation of ROS promotes oxidative stress that leads to lipid peroxidation, protein oxidation and DNA damage(Liu et al., 2020). Conversely, oxidative stress induced by AZ results in alterations in glutathione (GSH) redox status and inhibits antioxidant enzymes activity such as superoxide dismutase (SOD) and catalase (CAT Several clinical studies have demonstrated the hepatotoxic effects of AZ as well as experimental studies in vitro and in vivo(Abeer, 2015).Administration of AZ in experimental animals for 30 days with the double therapeutic dose results in increase the serum activities of liver function enzymes such as alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP)(Usadadia, 2020). El-kader (2020)demonstrated that marked increase in lipid

peroxidation product asmalondialdehyde (MDA) and decrease in activities of SOD, CAT and GPX in liver tissue after administration of AZ.

Luteolin (3′, 4′, 5′, 7′-tetrahydroxyflavone, Lut) is one of the most potent flavonoid biophenols that found in olive oil, peppermint, thyme, rosemary, and oregano(Lodhi et al., 2020). Lut has broad spectrum of pharmacological activities such as antioxidant, antidiabetic, anticancer and anti-inflammatory function(Zhang et al., 2017). Previous studies reported that Lut has protective effect against hepatotoxicity induced by carbon tetrachloride(Yan et al., 2019). Lut reduced proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukins (IL-6 and IL-1β)(Park and Song, 2019).In a previous experimental study, treatment with Lut to mice caused in decrease liver function activities such as ALT, AST and albumin levels in serum as compared with control mice(Kwon et al., 2015). The present study aimed to investigate the hepatoprotective effect of luteolin on azithromycin induced-hepatotoxicity in male rats.

Materials and methods

Chemicals:

Azithromycin (Xithrone 500 mg, Amoun pharmaceutical Co, Egypt) was purchased from local pharmacy. Luteolin was purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals and reagents were analytical grade or higher.

Animals:

The current study was performed in accordance with the international guidelines in laboratory animal care and use legislation, approved by a research ethics committee. Adult male wistar albino rats weighing 180-200 g were obtained from the Laboratory Animal Center, Veterinary Medicine school - Baghdad University - Iraq. Rats were kept in plastic cages with wood-ship bedding renewed every day and acclimatized to normal laboratorycondition for two weeks. Animals have been fed a rodent pellets diet and *ad libitum* water.

Experimental design:

After acclimatization, rats were divided randomly into four classes, each with ten animals:

1. **1. Rats in the control (Cont):**category were fed only a normal diet and were given tap water.
2. **Luteolin (LUT) group:** rats were givenorally LUT with the gastric tube (50 mg/kg.bw) for 4 weeks(Lodhi et al., 2020).
3. **Azithromycin (AZ) group:**rats were orally given AZ with gastric tube (30 mg/kg.bw) for 4 weeks(El-kader, 2020).
4. **Azithromycin (AZ) + Luteolin (LUT) group:**rats were orally given AZ (30 mg/kg.bw) and LUT (50 mg/kg.bw) for 4 weeks.

Obtaining samples

Fasted rats were anesthetized with ketamine/xylazine (0.1 ml/100g b.w ip) at the conclusion of the experiment time, and blood samples were obtained via hear puncture. Blood samples were centrifugated and serum kept at -20^{o} for biochemical analysis.After blood sampling immediately, liver from each rat were excised and cleaned. Portion of liver tissue was homogenized for biochemical assay. The other was preserved in formalin 10% for histopathological investigation.

Biochemical analysis:

Determination of liver function

Liver function aspartate aminotransferase (AST), alanine aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT) and albumin in the serum were determined using kits purchased from Spinreact (Girona, Spain).

Determination of oxidative stress markers and antioxidants

In the liver homogenate, malondialdehyde (MDA) levels was measured using BioDiagnostic Kit (Dokki, Giza, Egypt). The activities of superoxide dismutase (SOD) and catalase (CAT) and the levels of glutathione (GSH) in liver tissue were estimated using BioDiagnostic Kit (Dokki, Giza, Egypt).

Determination of pro-inflammatory cytokines

Interleukin 6 (IL-6), and interleukin 10 (IL-10) levels in serum were assessed using kits provided by BosterBio (California, USA).

Histological examination of liver sections

Liver specimens have been fixed in 10% buffered neutral formalin solution for 24 hours, dehydrated, and wrapped in paraffin. 5µ sections were stained with hematoxylin and eosin (H&E). The Slides were examined and photographed under a light microscope to detect the histopathological changes.

Statistical analysis

All data were statistically performed with GraphPad Prism7.02 software. Results are presented as mean ± SEM of n = 5. Statistical comparisons were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests.

Results:

Liver function tests:

Oral administration of AZ for 4 weeks shows significant increases in the serum activities of enzymatic liver function as ALT, AST and GGT with significant decrease in serum levels of albuminin comparison with control group. However, treatment with Lutshowed significant decreases in the serummentioned diagnostic markers of liver injury, as compared to AZ treated rats (table 1).

Table (1) Liver function for all groups

	Cont	Lut	AZ	AZ + Lut
ALT	21.45 ± 2.55	23.25 ± 1.90	76.29 ± 3.63***	37.46 ± 3.49*,###
AST	41.87 ± 2.98	42.81 ± 1.97	80.7 ± 1.99***	57.86 ± 3.94**,###
GGT	72.67 ± 3.17	73.48 ± 2.33	139.7 ± 4.92***	104 ± 5.65**,###
Albumin	11.04 ± 0.69	11.14 ± 0.86	3.143 ± 0.12***	7.1 ± 0.61**,###

Values are expressed as mean ± SEM of 5 rats per group.

* Significant at (P<0.05).

** Highly significant at (P<0.01).

*** & ### Very highly significant at (P<0.001).

*, **, *** Significant as compared with the control group.

Significant as compared with the Diabetic group.

Cont: Control, Lut: Luteolin, AZ: Azithromycin.

Oxidative stress marker and antioxidants in liver

The effect of Lut treatment on MDA content was measured to evaluate lipid peroxidation in Liver of all studied groups. The data showed a significant elevation in MDA levels in liver of AZ treated rats compared to control animals. The elevation in MDA level was significantly prevented by oral treatment with Lut compared with the AZ treated rats. For more instant, animals that received Lut alone showed no alteration in MDA in when compared to the normal control animals. In contrast, a significant depletion in GSH concentrations andactivities ofSOD, CATwas observed in liver of the AZ treated rats compared to control rats. On the other hand, Lut supplementation significantly prevented the depletion of GSH concentrations and activities of SOD, CAT in hepatic tissues of AZ treated rats treated with Lutcompared with untreated AZ treated rats (Fig. 1).

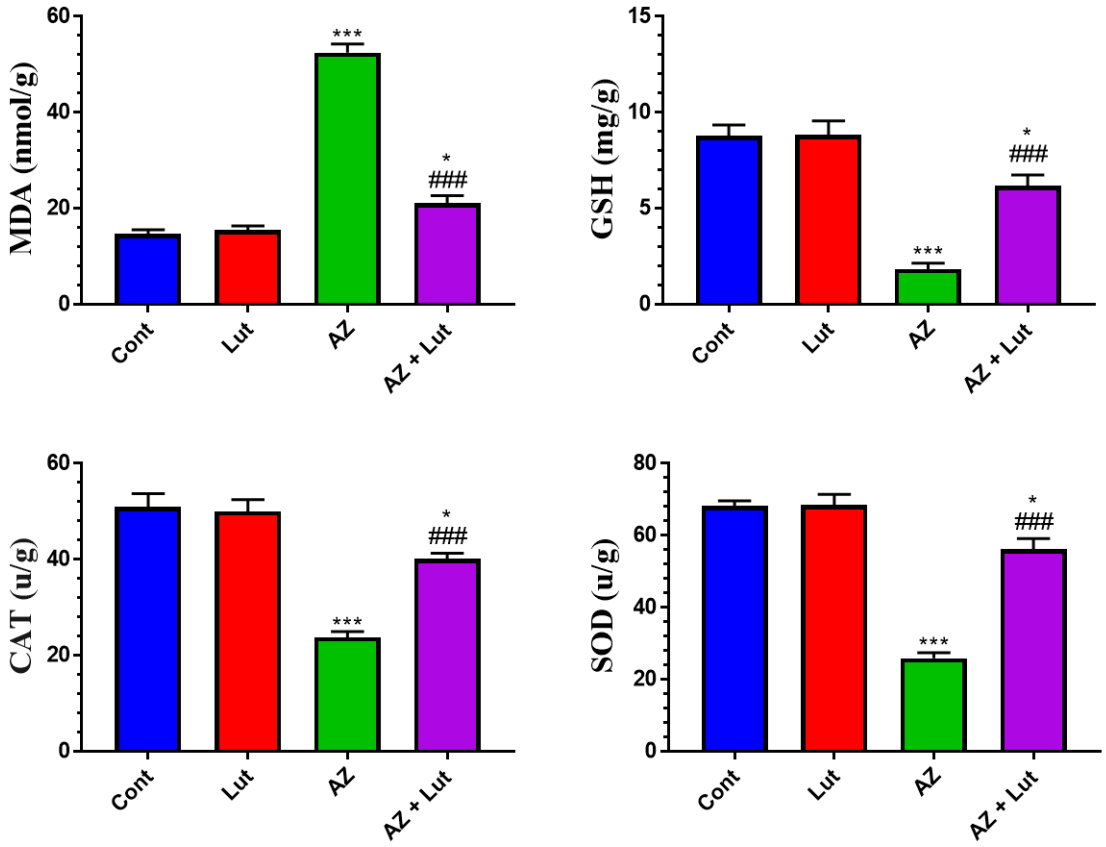

Fig. (1) Values are expressed as mean ± SEM of 5 rats per group.

* Significant at (P<0.05).

** Highly significant at (P<0.01).

*** & ### Very highly significant at (P<0.001).

*, **, *** Significant as compared with the control group.

Significant as compared with the Diabetic group.

Cont: Control, Lut: Luteolin, AZ: Azithromycin.

Pro-inflammatory and anti-inflammatory cytokines

AZ treated animals demonstrated a significant increase in IL-6 levels associated with a significant decrease in IL-10 level when compared with the control group. Luttreatment for 4 weeks significantly decreases the elevation in the pro-inflammatory mediator IL-6 and increase the anti-inflammatory cytokines (IL-10) compared to AZ treated rats (Fig. 2).

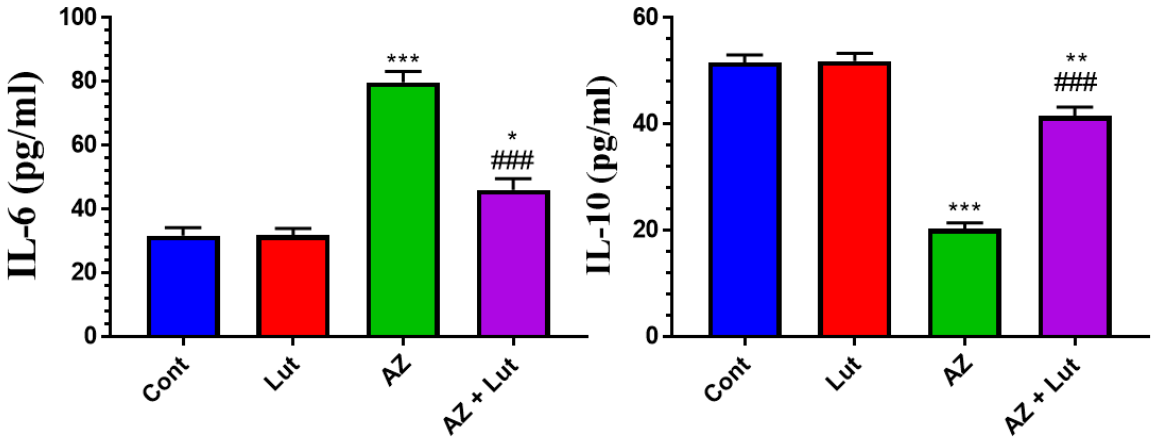

Fig. (2)Values are expressed as mean ± SEM of 5 rats per group.

* Significant at (P<0.05).

** Highly significant at (P<0.01).

*** & ### Very highly significant at (P<0.001).

*, **, *** Significant as compared with the control group.

Significant as compared with the Diabetic group.

Cont: Control, Lut: Luteolin, AZ: Azithromycin.

Livertissue histopathology:

Histological observation of liver tissues from control and Lut-only treated rats showed normal hepatocytes. In contrast, liver of AZadministrated animals showed hepatic damage, disarranged structure of hepatic lobules and vacuolation of hepatocytes. On the other hand, liver of AZ administrated animals treated with Lut showed nearly normal hepatocytes (Fig. 3).

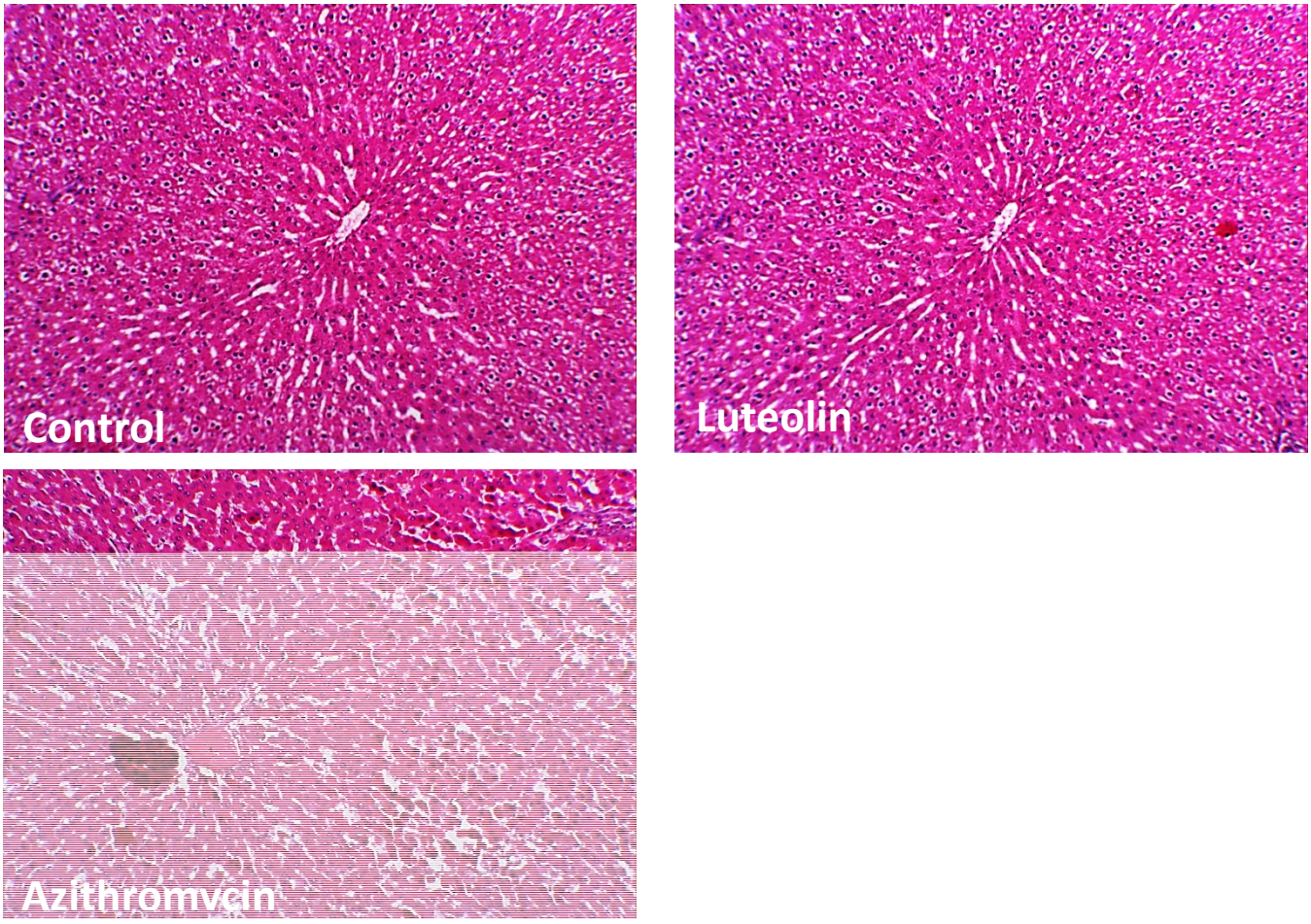

Fig. (3) Hematoxylin and eosin-stained liver sections(10X)

Discussion

Azithromycin is widely used as a second generation of macrolide antibiotic. However, accumulating evidence indicates that prolonged use of AZ increase risk of hepatotoxicity as elevation in activities of liver function enzymes and proteins(Martinez et al., 2015). The current study presents the hepatoprotective role of luteolinas a natural flavonoid against azithromycin induced hepatotoxicity through attenuation of oxidative stress and inflammation.

Treatment withluteolin prevented and ameliorated the liver damage and reserve histological integrity. Elevation levels of ALT, AST and GGT are the crucial markers for hepatic damage. The increase in ALT and AST enzymes activities reflect hepatic cell injury which intron associated with cell death(Center, 2007). GGT is a membrane bound enzyme found in the lining of the bile duct and the elevation of this enzyme indicate hepatobiliary disorders(Sharma et al., 2014). The present study revealed that serum of AZ-treated rats showed increased activities of ALT, AST and GGT with decrease in albumin levels. These results are in agreement with the earlier reports that demonstrate administration of AZ results in increase level of serum liver function enzymes and proteins resulted by loss of hepatic membrane architecture and cellular leakage(Lockwood et al., 2010, Li et al., 2016).

Treatment of AZ-treated rats with Lut displayed significant decrease in ALT, AST and GGT activities with increase in levels of albumin as compared with AZ-treated rats. This indicated the

improvement in architectural integrity of hepatocytes. In accordance with previous studies which reported potent hepatoprotective efficacy of Lut against hepatotoxicity of several toxins and drugs (Zhang et al., 2017, Cao et al., 2017, He et al., 2016).

Although the underlying mechanism by which AZ-induced hepatotoxicity are not fully understood, but could be ascribed to oxidative stress and inflammation. Popovic et al. (2008)reported that liver injury induced by AZ resulted from overgeneration of ROS which initiates lipid peroxidation process that leads to destruction of hepatocytes membrane results in release hepatic enzymes in blood.

Cytochrome P450 is kind of enzymes that regulate drug and xenobiotics metabolism in the liver (Zanger and Schwab, 2013). It has reported that generation of ROS and free radicalsduring metabolism of AZ by cytochrome P450. The oxidative stress resulted by ROS caused destruction of membrane components such as lipid and proteins that leads to form end products such as malondialdehyde (MDA) and protein carbonyl (Pc)(Mhadhbi et al., 2020).

The current data showed significant elevation of hepatic MDA content as lipid peroxidation product as well as significant decrease in hepatic activities of antioxidant enzymes as SOD and CAT, and GSH content in AZ-treated rats compared with control. These results in accordance with several studies reported that administration of AZ induced lipide peroxidation in hepatic tissue of rats(Li et al., 2016, Singh et al., 2016).

On the other hand, treatment of animals with Lut showed a significant depletion of MDA levels with increase in activities of SOD and CAT with elevation of GSH levels. These results reflect the increase of antioxidant defense system in liver tissue with decrease lipid peroxidation. This could explain the improvement in architectural integrity of hepatocytes and decrease in liver function enzymes. Several studies have demonstrated that oral supplementation of Lut ameliorate the elevation of the oxidative stress products and depletion in antioxidant defense system indicate its hepatoprotective activity(Shanmugam et al., 2016, Park and Song, 2019).

In a previous study, azithromycin administration affected expression levels of the cytokines IL-6(Sugiyama et al., 2007). Over production of ROS lead to oxidative stress which act as pro-inflammatory mediator that induce inflammation(Mittal et al., 2014). In agreement, our determinations showed significant increase in serum pro-inflammatory cytokine IL-6 as well as reduced in anti-inflammatory cytokine IL-10 in AZ-treated rats. It is widely accepted that free radicals induce inflammation which plays crucial role in the hepatotoxicity induced by AZ(Diego et al., 2013). This is in accordance with a study that AZ induced hepatic injury via over production of nitric Oxide (NO) in hepatic tissue that leads to activation inflammatory cytokines that lead finally in cell death(Gao et al., 2008). Conversely,Lut supplementation of AZ-treated rats showed significant increase in IL-10 with decrease in IL-6.

Accordingly, Shanmugam et al. (2016) reported that Lut inhibit the generation of proinflammatory cytokines. This in agreement with a study Lodhi et al. (2020) that demonstrated that Lut supplementation reduced the elevation in the levels of IL-1β, IL-6 and TNF-α.

The histopathological examination of liver tissue confirmed the biochemical data.Lut treatment to AZ-treated rats showed improvement in architectural integrity of hepatocytes as compared to AZ-treated rats.These findings coincided withZhang et al. (2017) who demonstrated that Lut reduce hepatocellular damage and restore the architectural integrity of hepatocytes

In conclusion, the current study demonstrated that luteolin may protect the liver against azithromycin-induced hepatotoxicity, oxidative stress and inflammation. The mechanism of luteolin

responsible for hepatoprotection involves restore the antioxidant defense system and suppress inflammation in hepatocytes. Luteolin also prevent the hepatocellular leakage and the increase in liver function enzymes. The protective role of luteolin on liver of rats was supported by histological investigation. Taken together, luteolin is as potent antioxidant, pharmacological, nutritional agent for ameliorating the hepatic damage and injury.

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