Hepatotoxic Effects of an Oral Amiodarone in White Albino Rats: Sub-Acute Biochemical, and Histopathological Assessments

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Article Info:

Received Feb 2023
Revised Apr 2023
Accepted May 2023
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DOI: https://doi.org/10.32947/ajps.v24i3.1058

Abstract:

Amiodarone, potent antidysrhythmic, widely used drug that has been associated with hepatic toxicity in long-term or excessive use. In the presented study twelve rats were allocated into two groups and given daily doses via gastric gavage orally for two weeks as follows;

The first group served as a control normal group, whereas the second got amiodarone at a dosage of 300mg/kg/day orally. Liver tissues were processed for light microscopy, and blood samples were examined for serum transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) and phospholipase a2 enzyme (Pla2) (which are thought to be an indicator of phospholipidosis and lipid buildup). Amiodarone was shown to produce hepatic histological abnormalities such as blood vessel congestion, leucocytic infiltration, liver degeneration, and stages of steatosis and necrosis of hepatocytes. The biochemical assessments were revealed that there was an elevation in amiodarone group's ALT and AST levels as a result of hepatic necrosis leading to leakage of enzymes content, while serum Pla2 was lowered significantly. Also, histopathology indicates stages of steatosis (Lipid accumulation) in hepatocytes, which may lead to farther damage in the late stages of hepatotoxicity.

Key words: amiodarone, hepatotoxicity, phospholipidosis.

التأثيرات السمية الكبدية للأميودارون عن طريق الفم في الجرذان البيضاء ألبينو: التقييمات البيوكيميائية والتشريحية المرضية دون الحادة

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الخلاصة:

الأميودارون، عقار قوي مضاد لاضطراب النظم، يستخدم على نطاق واسع ويرتبط بالتسمم الكبدي عند الاستخدام طويل المدى أو المفرط. في الدراسة المقدمة، تم تخصيص اثني عشر فأرًا إلى مجموعتين وتم إعطاؤهم جرعات يومية عن طريق أنبوب المعدة عن طريق الفم لمدة أسبوعين على النحو التالي: كانت المجموعة الأولى بمثابة مجموعة سيطرة عادية، في حين حصلت المجموعة الثانية على الأميودارون بجرعة 300 ملغم/كغم/يوم عن طريق الفم. تمت معالجة أنسجة الكبد لإجراء الفحص المجهري الضوئي، وتم فحص عينات الدم بحثًا عن الترانس امينيزيز في المصل (ناقلة أمين الألانين (ALT) وناقلة أمين الأسبارتات (AST)) وإنزيم الفوسفولايبيز(Pla2)، (التي يُعتقد أنها مؤشرات على داء الفسفوليبيدوز وتراكم الدهون). تبين أن الأميودارون يسبب تشوهات نسيجية كبدية مثل احتقان الأوعية الدموية، وارتشاح كريات الدم البيضاء، وتنكس الكبد، ومراحل التنكس الدهني ونخر خلايا الكبد. أظهرت التقييمات البيوكيميائية وجود ارتفاع في مستويات ALT وAST في مجموعة الأميودارون نتيجة النخر الكبدي الذي أدى إلى تسرب محتوى الإنزيمات، بينما انخفض مستوى Pla2 في الدم

بشكل ملحوظ. كما تشير التشريح المرضي إلى مراحل التنكس الدهني (تراكم الدهون) في خلايا الكبد، مما قد يؤدي إلى مزيد من الضرر في المراحل المتأخرة من السمية الكبدية.
الكلمات المفتاحية: أميوداورون ، سمية كبدية ، فوسفوليبيدوزيس.

Introduction

The liver is a target of toxicity. Because of its detoxifying role, that may lead to hepatotoxicity, which is a major health problem produced by many drugs and xenobiotics. [1,2] Amiodarone is one of these drugs which is class III an antiarrhythmic medication that is fre quent administered for a variety of conditions. It is a strong lipophilic, iodinated derivative of benzofuran [3]. Amiodarone was first developed as an antianginal agent in the 1960s. Despite the fact that it is licensed for the prevention of ventricular and atrial arrhythmias, treatment with amiodarone has been associated with a variety of reported and well-documented side effects, including those on the skin, thyroid, lungs (pulmonary fibrosis), cornea, and nerves, as a result of drug's accumulation in previous tissues [3,4]. It can cause steatosis in both animal models and people, probably by inhibiting mitochondrial B-oxidation of fatty acids via lysosomal and/or mitochondrial dysfunction, furthermore it can cause phosholipidosis by inhibiting phospholipases enzymes PLA1&PLA2 [5]. Apart from problems with pulmonary, thyroid, and nerve conduction effects, the possibility of amiodarone hepatotoxicity remains a serious issue. While rare, fatalities from amiodarone-induced hepatotoxicity have been documented [6]. Liver enzymes elevation which considered the primary sign of hepatotoxicity were reported in many cases after short and long periods of taking AMD. [7] There are two clinical manifestations of AMD hepatotoxicity: acute and chronic. The first occurs within 24 hours of receiving an intravenous, while another type occurs as a result of long-term oral treatment [8]. The exact mechanism of this drug to induce hepatotoxicity is not actually known because it can harm liver by different mechanisms, some previous studies showed that it can cause steatosis and phosholipidosis that may lead to cirrhosis (AIC death rates at 5 months might reach 60% [6]) with long durations of using amiodarone as antiarrhythmic drug, it could also cause necrosis or fibrosis [4].

Aim: The presented study aimed to assess the hepatotoxic effect of an oral amiodarone in white albino male rats through the histological; and biochemical analysis.

Materials and Methods

Study design: Twelve healthy adult male albino rats, weighed (200 –250 gm.) were used in the presented study. Animals were attained and placed in the animal house of the College of Pharmacy/ Mustinsiriyah University. The animals were kept for 1week under controlled circumstances of temperature (24 ± 2 C^0) and light (12-12 hours of light/dark cycle), allowed to access pellets and water freely, and located in plastic cages in dimensions (20x25x35 cm) that accommodated four rats. The animals in this experiment were divided into two groups with six rats in each one according to the followings: **Group 1 (n=6):** Negative control group; Normal group receive normal diet. **Group 2 (n=6):** Induction control group; given amiodarone (300mg/kg) [9] orally by gavage gage 8 for 2 weeks.

Biochemical analysis:

The reagents kits for the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and PLA2 were purchased from Sigma-Aldrich (St Louis, MO, USA) which measured by colorimetric (spectrophotometric) and Elisa methods. [10, 11]

Paraffin section preparation:
After two weeks of an oral amiodarone administration, the animals were anesthetized and scarified and liver tissue collected for histologic study. Formalin-fixed liver tissues were paraffin embedded, sectioned, and stained with hematoxylin and eosin (H&E). Light microscopy was used to analyze the stained liver slices.

Statistical analysis:
Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). Independent sample T-test were performed to assess significant differences among means. $P \leq 0.05$ is considered statistically significant [12].

Results:

The results of AST, ALT liver enzymes in the induction group are significantly highly increased ($p \leq 0.05$) compared to the control group (the healthy group with normal value) as shown in table (1) and figure (1). According to the results from the table (1-1), amiodarone significantly raises AST (mean=148.19) and ALT (mean=156.77). These values are significantly elevated in comparison to the healthy control group, which displayed much lower levels of liver enzymes (AST: 43.74; ALT: 29.50). The results of the preceding table (1-1) and figure (2) below demonstrated that amiodarone cause remarkable decrease in serum levels of pla2 (mean=1.83) in the induction (AMD) group, in comparison to the normal healthy group which showed a mean of 8.5. This means that AMD inhibit this enzyme by a specific mechanism

Table (1-1): the effect of AMD on liver enzymes and phospholipase A2

Biomarkers	Control (Mean±SE)	Induction (Mean±SE)
AST	43.74±1.10^{b}	148.19±12.23^{a}
ALT	29.50±3.65^{b}	156.77±14.27^{a}
PLA2	8.56±1.26^{a}	1.83±0.35^{b}

Data were expressed as means ± SE, SE: standard error of the mean. AST: aspartate aminotransferase, ALT: alanine aminotransferase, pla2: phospholipase A2 enzyme. The different small letters show the significant differences between groups, $P \leq 0.05$ indicate a significant difference

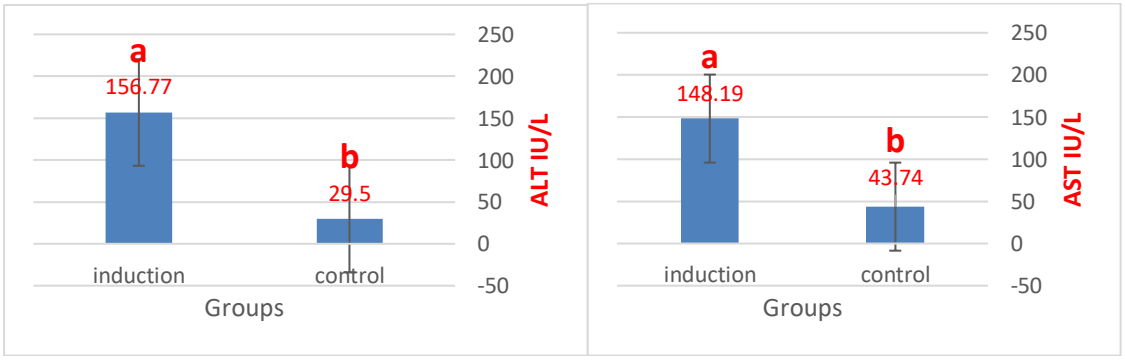


Figure (1): Effect of amiodarone on AST, ALT liver enzymes activity.
The different small letters (a, b) show the significant differences between groups. Induction group: amiodarone group, control: normal group

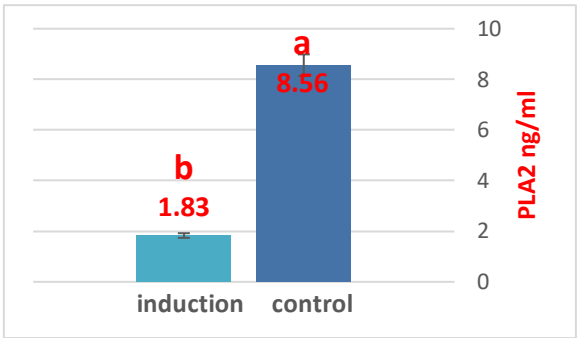


Figure (2): Effect of amiodarone on phospholipase enzyme a2 activity.

The different small letters (a, b) show the significant differences between groups. Induction group: amiodarone group, control: normal group

Histopathological study:

histoicrograph of liver of control group showed normal arragment of hepatic cords, normal central veins, siunsoids & kupffer cells,no signs of inflammations and necrosis or tissue degenerations (figure 3&4).Liver's sections in induction group showed moderate dilation with congestion of the central veins, sinusodal congestion and multiple focal tissue depletion (figure5). The magnified sections revealed stages of liver stetosis (lipid accumulations) (figure6). Other section revealed advanced vacular degeneration and necrosis of most hepatocytes and marked inflammatory cells clustering , the affected hepatocytes revealed nuclear pyknosis and other revealed karyorrehxes (figure7&8).

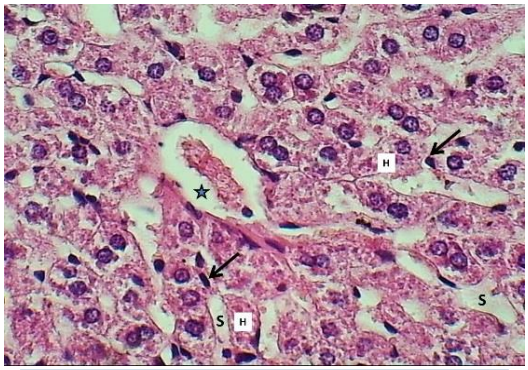


Figure 3: section of liver (Control) shows: Central vein (arrows) & hepatic cords (Asterisks). H&E stain.400x.

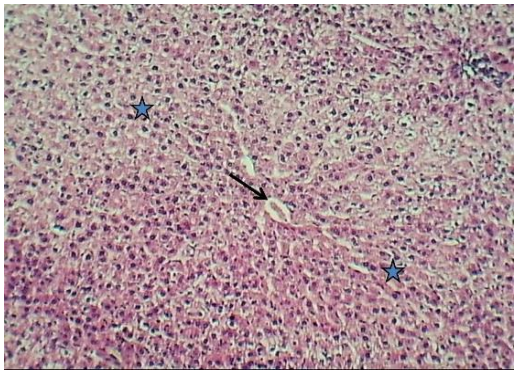
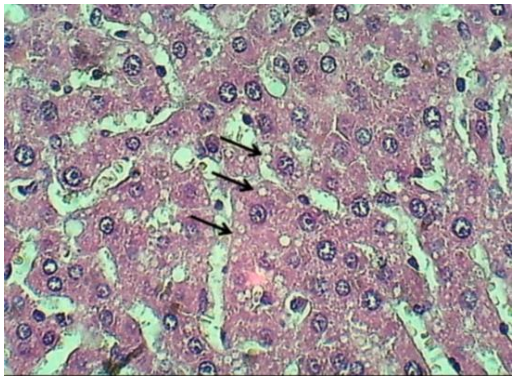


Figure 4: Section of liver lobule (Control) shows: normal central vein (asterisk), hepatocyte (H), sinusoid (S) & kupffer cells (Arrow). H&E stain.400x



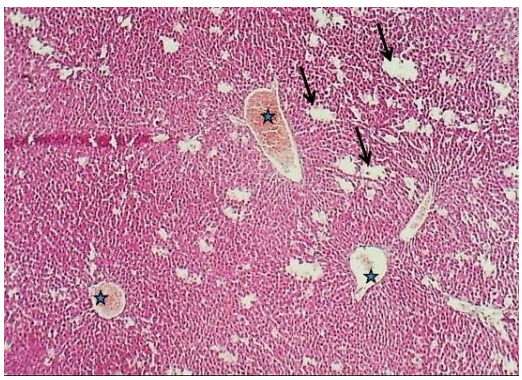


Figure 5: section of liver (induction) shows: dilation and congestion of central vein (Asterisks) & focal necrosis and tissue depletion (Arrows). H&E stain.400x.

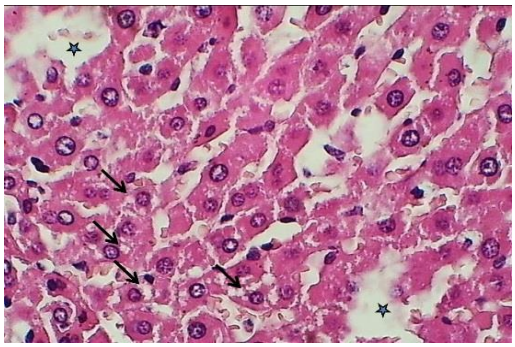


Figure 6: section of liver (induction) shows: marked liver steatosis (Black arrows) of hepatocytes and sinusoidal congestion. H&E stain.400x

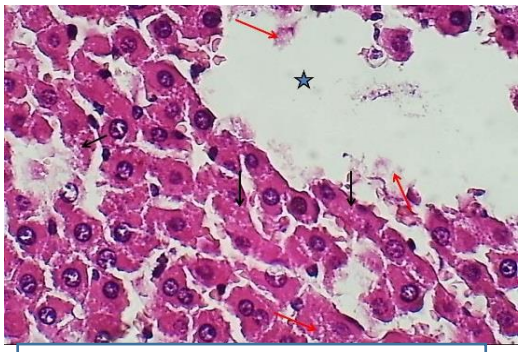


Figure 7: section of liver (induction) shows: steatosis (degeneration) (Black arrows) and necrosis (Red arrows) of hepatocytes and tissue depletion (Asterisks). H&E stain.400x

Figure 8: section of liver (induction) shows: sever degeneration (Black arrows) of hepatocytes and tissue depletion (Asterisks). H&E stain.400x

Discussion

The findings obtained from the presented study demonstrated that AMD causes histological changes in rat hepatic tissue. Hepatic cell necrosis was detected along with liver sections in the toxic induction group; some of the hepatocytes were vacuolated with significant damage related with central vein. The presented study was agreed with Fonseca et al. (2015) [13]. The necrosis of membranes caused by the generation of ROS and oxidative stress results in the leaking of enzymes from cells. [14].Therefore significant elevation of serum ALT and AST enzyme activities in induction group indicates liver cells damage. According to previous research, AMD administration by gavage for four days had the same effect on liver damage as an 11-day administration [15]. In the presented study, the 14-days regimen of AMD administration orally in dose of 300g/kg/day led to significantly increased levels of liver enzymes and also caused hepatic steatosis (Figure 5, 6). Moreover, AMD inhibits mitochondrial beta-oxidation of fatty acids, resulting in their accumulation in liver tissues and liver steatosis [3]. Furthermore, the serum levels of PLA2 were markedly decreased and inhibited as a result of amiodarone accumulation in the lysosomes of hepatocytes, which block phospholipases A1 and A2 as well as hinder the elimination of lysosomal lipids and lead to phospholipidosis [16, 17].

Conclusion

The findings obtained from the presented study suggested that AMD Induce hepatotoxicity by destruction of hepatocytes and increase in serum enzymes of ALT, AST and inhibition of the enzyme PLA2 that responsible for metabolism of phospholipids, this result in PLD and steatosis (lipid accumulation) in liver and further damage occur. histological and morphological changes revealed advanced vacular degeneration and necrosis of most hepatocytes,inaddition to steatosis (degeneration).

Acknowledgement

The authors would like to thank the Deanship of Pharmacy College and the chairman of pharmacology and toxicology department at College of Pharmacy/ Mustinsiryiah University for giving their support.

Abbreviations
AMD amiodarone.
PLA2 phospholipaseA2.
PLD phospholipidosis.
AST aspartate aminotransferase.
ALT alanine aminotransferase.
AIC amiodarone induced cirrhosis

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