

## The Hepatoprotective Effect of *Glycyrrhiza glabra* Roots Extract Against Amiodarone Induced Hepatotoxicity in Male Rats.

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### Abstract

Amiodarone, a powerful antidysrhythmic medication, can cause hepatotoxicity when used long-term or in high doses. It affects both mitochondrial and lysosomal function because of its lipophilic nature and accumulation in tissues. *Glycyrrhiza glabra* is one of the medicinal plants that have been commonly used for thousands of years. It possesses anti-inflammatory and antioxidant properties. In this study, thirty rats were taken and divided into five groups given daily doses given orally by gastric gavage as follows: the first group acted as a normal control given distilled water and DMSO. And the second group received amiodarone at a dose of 300 mg/kg dissolved in DMSO for 2 weeks. The last three groups were pretreated with *Glycyrrhiza glabra* in doses of 200, 400, and 800 mg, respectively, for one week, and then with amiodarone (300mg) for 2 weeks. Hepatic damage, revealed by histology and the increased activities of serum aspartate aminotransferase and alanine aminotransferase and decreased serum phospholipase A2 in the amiodarone group, *Glycyrrhiza glabra* extract significantly reduced liver enzymes, restoring PLA2 to its normal level. It also enhanced liver superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde levels. Moreover, *Glycyrrhiza glabra* extract markedly decreases serum TNF levels, demonstrating its anti-inflammatory, antioxidant, and hepatoprotective actions.

**Keywords:** *Glycyrrhiza glabra*, Amiodarone, Drug induced liver injury, Hepatotoxicity, Phospholipidosis.

### التأثير الوقائي الكبدي لمستخلص جذور عرق السوس ضد السمية الكبدية المستحثة بالأميودارون في ذكور الجرذان.

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#### الخلاصة

الأميودارون، وهو دواء قوي مضاد لاضطراب النظم، يمكن ان يسبب تسمم الكبد عند استخدامه على المدى الطويل أو بجرعات عالية. إنه يؤثر على كل من وظيفة الميتوكوندريا والليزوسومات بسبب طبيعته المحبة للدهون والتراكم في الأنسجة. عرق السوس هو أحد النباتات الطبية التي تم استخدامها بشكل شائع لآلاف السنين. يمتلك خصائص مضادة للالتهابات ومضادة للأكسدة. في هذه الدراسة تم أخذ ثلاثين فأراً وقسمت إلى خمس مجموعات أعطيت جرعات يومية عن طريق الفم بوساطة الأنبوب المعدي على النحو التالي: المجموعة الأولى عملت كمجموعة طبيعية أعطيت الماء المقطر و DMSO. وتناولت المجموعة الثانية الأميودارون بجرعة 300 مجم / كجم لمدة أسبوعين كمحلول مذاب بال DMSO. تمت معالجة المجموعات الثلاث الأخيرة بعرق السوس بجرعات 200 و 400 و 800 مجم على التوالي لمدة أسبوع، ثم بالأميودارون م (300 مجم) مع عرق السوس لمدة أسبوعين. تم الكشف عن الضرر الكبدي من خلال الأنسجة وزيادة نشاط مصل الأسبارتات أمينوترانسفيراز والأنين أمينوترانسفيراز وانخفاض فوسفوليباز المصل 2A في مجموعة الأميودارون، مستخلص عرق السوس قلل بشكل كبير من إنزيمات الكبد، وأعاد PLA<sub>2</sub> إلى مستواه الطبيعي. كما أنه عزز مستويات السوبر اوكسايد ديسموتاز في الكبد (SOD) والجلوتاثيون (GSH) ومستويات مالونديالدهيد. علاوة على ذلك، مستخلص عرق السوس قلل بشكل ملحوظ من مستويات TNF في الدم، مما يدل على تأثيره المضاد للالتهابات ومضادات الأكسدة والتأثير الوقائي الكبدي.

الكلمات المفتاحية: عرق السوس، أميودارون، الادوية المسببة لاصابة الكبد، السمية الكبدية، داء الفسفوليبيد.

### Introduction

Drug-induced liver injury is a condition that can be brought on by a variety of medications and chemicals. These medications can induce a wide range of complications. (1, 2). One of them is amiodarone, an effective, widely used class III antiarrhythmic medication. (3) Amiodarone (AMD)

is a cationic lipophilic compound that accumulates in tissues, especially the liver, affecting both mitochondrial and lysosomal function. It inhibits phospholipase enzymes (PLA1 and PLA2), causing phospholipidosis that may lead to necrosis due to leakage of proteolytic enzymes from aberrant

lysosomes. Moreover, it impairs mitochondrial B oxidation, causing steatosis and oxidative stress<sup>(4)</sup>. *Glycyrrhiza glabra* (Gg) is one of the medicinal plants that have been commonly utilized in medicine for thousands of years by ancient Assyrian, Greek, Roman, Egyptian, Chinese, and Indian cultures for liver, gastrointestinal problems, cough, bronchitis, and arthritis.<sup>(5)</sup> The roots of this plant are the most important part as a result of their high content of pharmacologically active ingredients that are widely used in medicine. Glycyrrhizin, a triterpenoid saponin compound (50 times sweeter than sucrose), is the major bioactive compound in liquorice root and has many pharmacological activities. The Gg possesses anti-ulcer, anti-inflammatory, antioxidant, antimalarial, expectorant, antispasmodic, diuretic, laxative, and sedative properties.<sup>(6)</sup> This study aimed to investigate the hepatoprotective effect of *Glycyrrhiza glabra* roots extract against amiodarone hepatotoxicity in male rats by studying the biochemical and histological changes.

## Materials and Methods

### Study design

Thirty non-previously treated male albino rats, weighing 200–300 g, were provided from the Iraqi Center for Cancer and Medical Genetics Research (ICCMGR), Mustansiriyah University. The study took place in Mustansiriyah University / pharmacy department, and according to the Ethical Committee on Animal Care (file No. 17 in 14 November 2021), the animals were housed in a well-ventilated place with woodchip bedding, maintained under standard conditions of relative humidity ( $70 \pm 5\%$ ), temperature ( $25 \pm 2^\circ\text{C}$ ), and a 12-hour light-dark cycle. To determine the effect of *Glycyrrhiza glabra* extract as a hepatoprotective agent, the groups in this study were divided into five groups with six rats in each group according to the following: Group 1 (n = 6): negative control group; normal group receives a normal diet and DMSO. Group 2 (n = 6): Induction control group; given amiodarone (300 mg/kg dissolved in DMSO) orally (by dose according to the previous study)<sup>(7)</sup> for 2 weeks. Group 3 (n = 6): Gg root (200 mg/kg) given orally for 1 week as a protection, then together with amiodarone (200/300mg) for 2 weeks. Group 4 (n = 6): Gg root (400 mg/kg) given orally for 1 week as a protection, then together with amiodarone (400/300mg) for 2 weeks. Group 5 (n = 6): Gg root (800 mg/kg) given orally for 1 week as a protection, then together with amiodarone (800/300mg) for 2 weeks. All the animals were anesthetized on the last day of the experiment, and blood samples were collected from the right ventricle of the heart by using a 5 mL syringe and then centrifuged to separate the serum.

### Chemicals and drugs

Amiodarone hydrochloride was purchased from Amin Pharmaceutical Company (Isfahan, Iran) with a purity > 99.9%, prepared as a stock solution by dissolving it in DMSO (10 mg/1 ml), and ingested by oral gavage gage 8. *Glycyrrhiza glabra* root extract was purchased as standard from Sigma-Aldrich (St. Louis, MO, USA).

### Biochemical analysis

The biochemical parameter measured serum alanine aminotransferase, aspartate aminotransferase, oxidative stress biomarker (glutathione, malondialdehyde, superoxide dismutase), tumor necrosis factor Alfa, and phospholipase A2 were purchased from Sigma-Aldrich (St Louis, MO, USA) and MyBioSource, USA that measured by colorimetric (spectrophotometric) and ELISA methods.<sup>(8, 9, 10, 11, 12)</sup>

### Preparation of paraffin section

At the end of the third week of the experiment, the animals were anesthetized for histologic preparations. Formalin-fixed liver samples were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) staining. The stained Liver sections were examined using light microscopy.

### Statistical analysis

Statistical analysis of data was performed using SAS (Statistical Analysis System - version 9.1). One-way ANOVA and Least significant differences (LSD) post hoc test were performed to assess significant differences among means.  $P \leq 0.05$  is considered statistically significant<sup>(13)</sup>.

## Results

The results from below table showed that the means serum levels of AST and ALT were significantly different ( $P \leq 0.05$ ) among all of the studied groups. Moreover, rat's amiodarone group caused a significant elevation in mean level of serums AST& ALT, compared with the normal control group. All the treatment groups demonstrated significant decrease in AST, ALT serums levels compared with induction group. Additionally, there is a significant difference between all treatment groups, on the other hand, treatment group 3 (800mg) displaying the best outcome due to the greatest significant decrease in AST&ALT serum levels. The results of TNF- $\alpha$  estimated significant differences between all groups  $P \leq 0.05$ , it can be seen that the induction group demonstrated a significant increase in serum TNF- $\alpha$  ( $242.89 \pm 48.85$ ) compared to the control and treatment groups. Additionally, the significant difference between treatment group 3 800mg ( $20.45 \pm 5.38$ ) and treatment group 1 200mg ( $99.31 \pm 13.67$ ) showing that this larger dose of plant extract had the greatest impact on decreasing TNF $\alpha$  levels and inhibiting inflammation. An inhibitory effect of amiodarone on phospholipaseA2 enzyme was

indicated by the significantly lower level of rat's PLA2 serum levels in the induction group as compared to controls. The *Glycyrrhiza glabra* roots extract treatment group 3 (800 mg) demonstrated a significant elevation of PLA2 level ( $7.01 \pm 0.66$ ) compared to the induction ( $1.83 \pm 0.35$ ), treatment group 1, and treatment group 2. The results estimated that the level of GSH in serum  $16.71 \pm 0.67$  of rat's liver are significantly decreased in the induction group as compared with the control normal ( $59.33 \pm 9.89$ ) group. Treatment group 3 remarkably exceeded the induction group ( $48.42 \pm 5.60$ ). The data of blood serum oxidative biomarkers are reported in Table (1), SOD of amiodarone-treated group rats was lower than in control normal one, showing oxidative effects of AMD. Treatment groups 1, 2 and 3 showed significant increase in

serum SOD in dose dependent manner especially in the treatment group 3 (800 mg) which gave the highest value ( $85.68 \pm 4.63$ ). Malondialdehyde level is commonly known as a marker of lipid peroxidation, in comparison with the control group, the induction AMD group showed a significant increase in serum MDA level, this increment is reversed by treatment groups (*Glycyrrhiza glabra* + AMD). All the treatment groups were significantly different from each other and induction group; serum MDA was highly decreased in treatment group 3 ( $0.81 \pm 0.02$ ), which differed significantly from the control normal group ( $1.58 \pm 0.19$ ). Also, MDA rat's serum levels were normalized in treatment group 2 (400 mg):  $1.57 \pm 0.25$ .

**Table 1. The mean $\pm$ SD of biomarkers in all study groups**

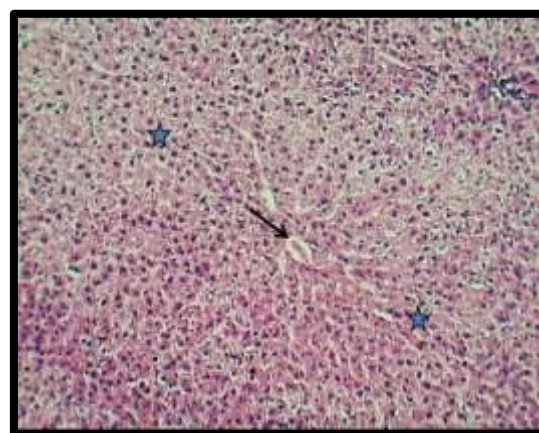
NO.	Groups name	AST mean $\pm$ SD	ALT mean $\pm$ SD	GSH mean $\pm$ SD	MDA mean $\pm$ SD	SOD mean $\pm$ SD	TNF $\alpha$ mean $\pm$ SD	PLA2 mean $\pm$ SD
G1	Control	43.74 $\pm 1.10^c$	29.50 $\pm 3.65^c$	59.33 $\pm 9.89^a$	1.58 $\pm 0.19^c$	67.90 $\pm 6.01^b$	43.80 $\pm 6.58^{bc}$	8.56 $\pm 1.26^a$
G2	Induction	148.19 $\pm 12.23^a$	156.77 $\pm 14.27^a$	16.71 $\pm 0.67^b$	4.88 $\pm 0.26^a$	24.25 $\pm 2.81^c$	242.89 $\pm 48.85^a$	1.83 $\pm 0.35^b$
G3	Treatment group 1	80.15 $\pm 2.67^b$	91.42 $\pm 10.87^b$	21.29 $\pm 2.29^b$	2.65 $\pm 0.40^b$	37.50 $\pm 4.20^c$	99.31 $\pm 13.67^b$	2.57 $\pm 0.89^b$
G4	Treatment group 2	31.57 $\pm 1.61^{cd}$	31.19 $\pm 5.19^c$	24.95 $\pm 5.07^b$	1.57 $\pm 0.25^c$	65.63 $\pm 5.12^b$	58.20 $\pm 0.93^{bc}$	4.15 $\pm 0.61^b$
G5	Treatment group 3	24.21 $\pm 2.06^d$	18.17 $\pm 1.40^c$	48.42 $\pm 5.60^a$	0.81 $\pm 0.02^d$	85.6 $\pm 4.63^a$	20.45 $\pm 5.38^c$	7.01 $\pm 0.66^a$
	LSD	16.72	24.86	16.51	0.75	13.63	68.24	2.33

AST: aspartate aminotransferase, ALT: alanine Aminotransferase, PLA2: phospholipase A2, GSH: glutathione, SOD: superoxide dismutase, MDA: malondialdehyde. LSD: least significant differences. The different superscript shows the significant differences between groups,  $P \leq 0.05$  indicates a significant difference.

#### Histopathological analysis study

The sections of liver of normal control group showed a normal tissue architecture (Figure.1&2). The sections of liver from Induction group showed sinusoidal congestion and multiple focal tissue depletion (Figure.3). The magnified sections revealed stages of liver steatosis (Figure 4). Other section revealed advanced vacular degeneration and necrosis of most hepatocytes and marked inflammatory cells clustering (Figure 5 & 6). The sections of liver for treatment group 1

showed mild disarrangement of hepatic cords and sinusoidal dilation (Figure.7). The magnified sections were revealed that most hepatocytes were normal with few Figures of steatosis and necrosis (Figure 8). Treatment group 2 and treatment group 3 were similar to group of normal control which showed normally arranged hepatic cords, normal central vein and normal appearance of hepatocytes (Figure 9,10,11,12).



**Figure 1. section of liver (Control) shows Central vein (arrows) & hepatic cords (Asterisks). H&E stain.100x.**



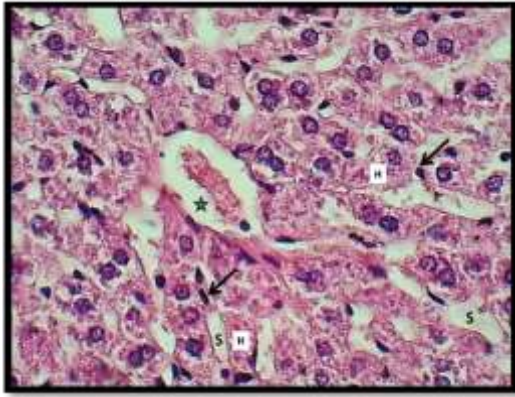


Figure 2. Section of liver lobule (Control) shows normal central vein (asterisk), hepatocyte (H), sinusoid (S) and kupffer cells (Arrow). H&E stain.400x

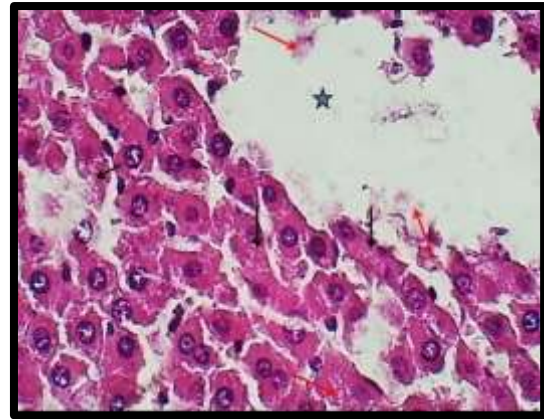


Figure 5. Section of liver (induction) shows: steatosis (degeneration) (Black arrows) and necrosis (Red arrows) of hepatocytes and tissue depletion (Asterisks). H&E stain.400x

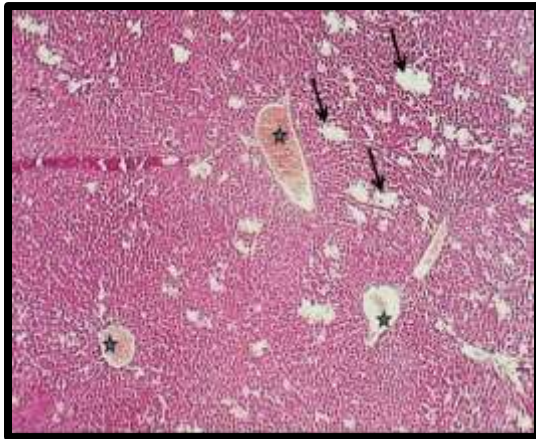


Figure 3. Section of liver (induction) shows dilation and congestion of central vein (Asterisks) & focal necrosis and tissue depletion (Arrows). H&E stain.100x.

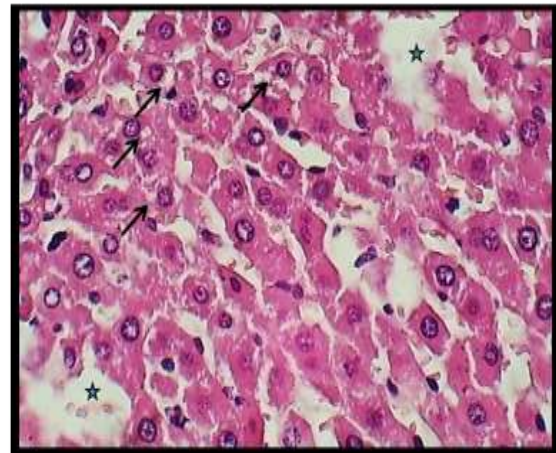


Figure 6. Section of liver (induction) shows: sever degeneration (Black arrows) of hepatocytes and tissue depletion (Asterisks). H&E stain.400x

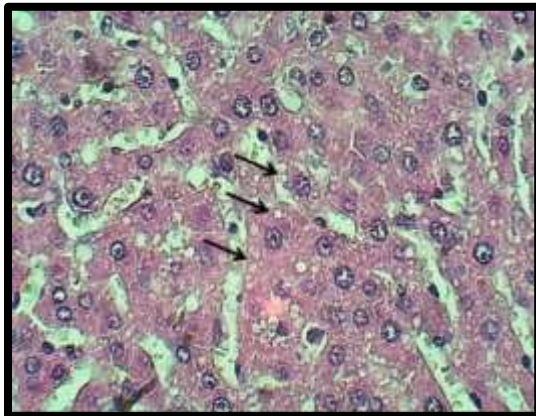


Figure 4. Section of liver (induction) shows: marked liver steatosis (Black arrows) of hepatocytes and sinusoidal congestion. H&E stain.400x

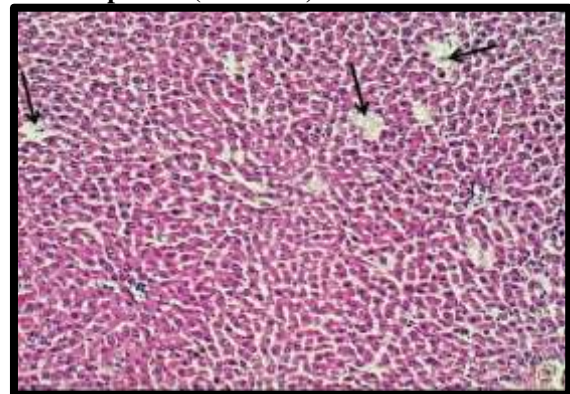


Figure 7. section of liver (G1-200mg) shows dilation of sinusoid with few & focal tissue depletion (Arrows). H&E stain.100x.



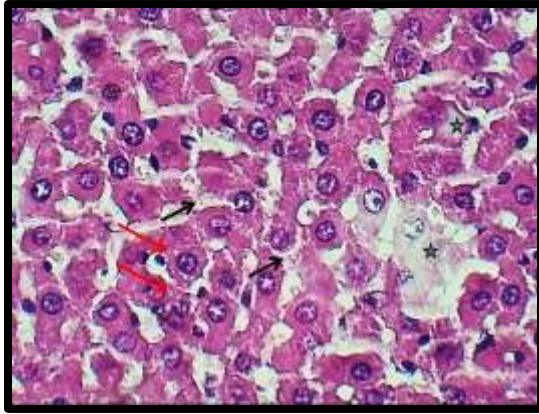


Figure 8. Section of liver (G1-200mg) shows: mild steatosis (Red arrows), necrosis (Asterisk) & mild sinusoidal congestion (Black arrows). H&E stain.400.

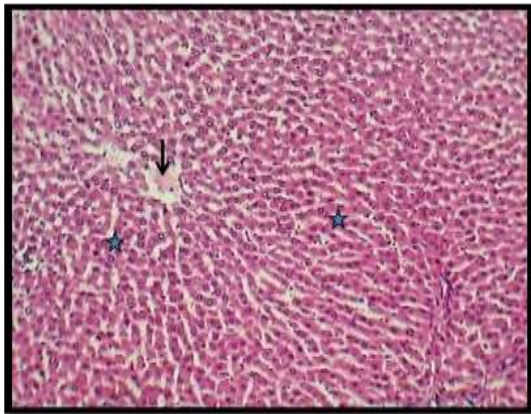


Figure 9. Section of liver (G2-400mg) shows: normal central vein (Arrow) & hepatic cords (Asterisks). H&E stain.100x.

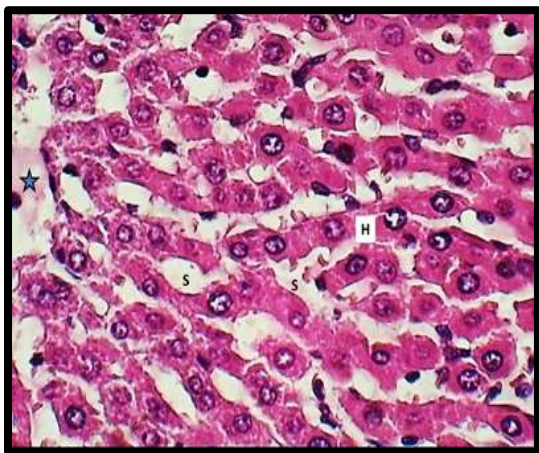


Figure 10. Section of liver (G2-400mg) shows normal central vein (asterisk), hepatocyte (H), sinusoid (S). H&E stain.400x.

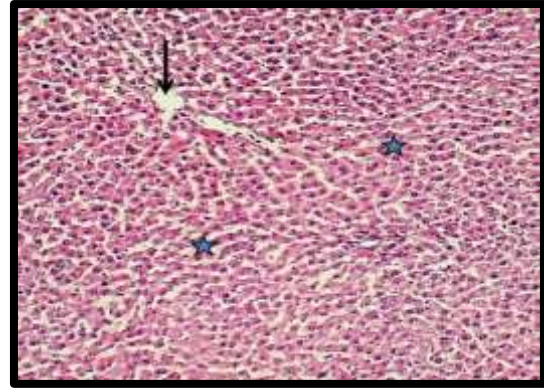


Figure 11. Section of liver (G3-800mg) shows: normal central vein (Arrow) & normally arranged hepatic cords (Asterisks). H&E stain.100x.

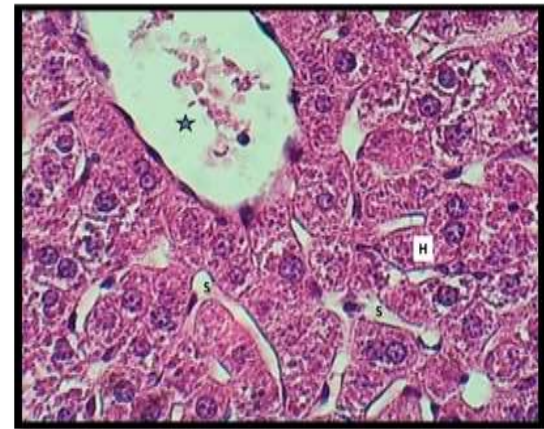


Figure 12. Section of liver (G3-800mg) shows normal appearance of central vein (Asterisk), sinusoids (S) & hepatocytes (H). H&E stain.400x

## Discussion

Current study show that amiodarone caused massive liver damage, as seen by significantly elevated serum ALT and serum AST activities. This result aligns with Li et al. (2015),<sup>(14)</sup> they found that intravenous administration of amiodarone caused an increase in ALT and AST serum levels. Moreover, Abdul-Hamid et al. (2018), investigated the effects of orally administering amiodarone to groups of albino rats, which led to liver damage, leakage of liver enzymes from destroyed hepatocytes, triggered an inflammatory reactions<sup>(15)</sup>. TNF $\alpha$ , a proinflammatory biomarker, was significantly higher in the group treated with amiodarone, demonstrating inflammation and necrosis in hepatic tissue.<sup>(16)</sup> Histopathological examination of the liver revealed signs of enlargement and degeneration, this was accompanied by steatosis, severe centrilobular necrosis, and liver cell infiltration by inflammatory cells Figure 3, 4, 5, and 6<sup>(17)</sup> Interestingly, Glycyrrhiza glabra root extract given before and along with amiodarone counteracts the drug's hepatotoxic effects due to their antioxidant and anti-inflammatory activities by bringing serum liver

enzymes to the normal range in treatment groups 2 and 3. This result was consistent with earlier research, which determined that the active ingredients 18-glycyrrhizin in Gg were responsible for the hepatoprotective effect of it by lowering liver enzymes in a group of rats intoxicated with CCl<sub>4</sub>.<sup>(18)</sup> Due to its vast volume of distribution and lipophilic nature, amiodarone accumulates in lipid-rich reservoirs, modifying membrane permeability and causing damage to the liver tissues as a result of the direct production of ROS and oxidative stress. Amiodarone cause impairment of cellular respiration, mitochondrial B-oxidation and decoupled oxidative phosphorylation.<sup>(19)</sup> The current study revealed that AMD significantly raised MDA level, decreased SOD and GSH levels in rats' liver tissue and serum in the induction AMD group (300 mg/kg) in comparison with the control group. These findings corroborated those of Saleem et al. (2016), who investigated how Crocus sativus counterd Amiodarone's hepatotoxic activity and showed that AMD depletes the liver's GSH reservoir, causing oxidative stress and liver damage.<sup>(20)</sup> The pre & co-administration of Gg extract with AMD normalized serum level levels of GSH, SOD and MDA, protecting the liver from oxidative stress. Furthermore, The Antioxidant activity of the extract of *Glycyrrhiza glabra* was determined, using the hydroxyl radical scavenging assay in an in vitro study done by Varghese et al. (2020),<sup>(21)</sup> it was indicated that the extract possesses free radical scavenging activity in a dose-dependent manner, restoring GSH levels to normal and inhibiting lipid peroxidation. Adil et al. (2021), also discovered that pre administration of licorice extract raised GPx, GSH, and catalase levels as well as their activities in the testis and serum of mice intoxicated with MTX injections<sup>(22)</sup>. The latter researches supported the findings of the present study. Lysosomal phospholipase A is essential for the degradation of lysosomal glycerophospholipids in mammalian cells, and phospholipidosis occurs as a result of its inhibition.<sup>(23)</sup> AMD and its key metabolite, desethylamiodarone, have been shown to bind phospholipase A2, and inhibiting it<sup>(24)</sup>, as shown in this study, serum PLA2 levels was reduced in the AMD induction group. Gg extract prevents PLD when administered with AMD, giving protection against lipid accumulation (steatosis & PLD) in liver.<sup>(25)</sup>

## Conclusion

*Glycyrrhiza glabra* root extract is a commonly used medicinal plant, it is rich in phytochemicals that provide antioxidant and anti-inflammatory properties. The current study found that Gg has a significant hepatoprotective impact by suppressing amiodarone-induced liver damage and modulating liver enzymes, TNF $\alpha$ , and oxidative stress indicators, restoring them to normal and

avoiding histological degeneration, steatosis, and necrosis.

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## Conflicts of Interest

There is no Conflict of interest regarding the publication of this manuscript.

## Funding

There is no financial support from any institution.

## Ethics Statements

Animals that used in this study were housed according to the Ethical Committee on Animal Care (file No. 17 in 14 November 2021) from Mustansiriyah University / pharmacy department.

## Author Contribution

Study conception and design: alzharaa Fatima safa'a, yasir Mustafa kamal, huda jaber waheed; Data collection: alzharaa Fatima safa 'a; Analysis and interpretation of results : alzharaa Fatima safa 'a; Draft manuscript preparation : alzharaa Fatima safa 'a; All authors reviewed the results and approved the final version of the manuscript.

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