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Protective Effect of *Zizyphus spina christi*-Leaves Extract against Amiodarone-Induced Hepato - and Nephrotoxicity in Male Albino Rats

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Abstract

Amiodarone (AMD), an orally potent antidysrhythmic medication that has caused hepato-renal toxicity on long-term administration, is commonly used across the world. *Zizyphus spina christi* leaves extract have ameliorative effects (ZSC). This research elucidated the magnitude of the damage to the liver and kidney tissues in AMD. Twenty four male albino rats were grouped into three groups given daily doses by gastric tube for two weeks as follows; the 1st group acted as a control group; the 2nd group received AMD; the 3rd group received AMD parallel to ZSC. Liver and kidney tissues prepared for histological examination and serum samples screened for biomarkers including; (I) liver injury enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP); (II) kidney injury indicators: urea, creatinine and albumin; and (III) inflammatory markers: interleukins (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α). The findings showed that AMD caused hepatic and renal histological changes that included congestion of the blood vessels, leucocytic infiltration, dilated bile ducts and lymphatics and cytoplasmic vacuolation were observed. The biochemical findings showed an increase in the AMD group's ALT, AST, ALP, urea and creatinine levels in addition decrease in albumin concentration. The group of rats treated with AMD and ZSC, increased the improvements in liver and kidney histology, while the ALT, AST, ALP, urea and creatinine levels were reduced and albumin level increased and ameliorative the levels of inflammatory markers, interleukins. This finding collectively agreed that ZSC has a protective impact on AMD hepato-nephrotoxicity which can be due to its antioxidant properties.

Keywords: Amiodarone; Cytokines; Hepatotoxicity; Nephrotoxicity; *Zizyphus spina Christi*

List of abbreviation

AMD: Amiodarone
ALT: Alanine aminotransferase
ALP: Alkaline Phosphatase
IL-1α: Interleukin 1 alpha
IL-4: Interleukin 4
IL-10: Interleukin 10
IFN-α: Interferon alfa
P.O: Per oral
CV: Central venule
Cap: Capsule
PT: Proximal convoluted tubules
ZSC: *Zizyphus spina Christi*
AST: Aspartate transaminase
ALBU: Albumin
IL-1β: Interleukin 1 beta
IL-6: Interleukin 6
IFN-γ: Interferon gamma
ROS: Reactive Oxygen Species
ELISA: enzyme-linked immunosorbent assay
BS: Blood sinusoids
BC: Bowman's capsule
DT: Distal convoluted tubules

1. Introduction

Amiodarone is one of the antiarrhythmic medications most commonly and widely prescribed for the treatment of ventricular and supraventricular tachyarrhythmias (Nasser *et al.,* 2013). A highly successful medication for the treatment of some cardiac arrhythmias, amiodarone is a benzofuran derivative that plays a significant role in the care of patients with both atrial and ventricular fibrillation (Roy *et al.,* 2000). AMD has a long half-life and a weak bioavailability and tends to accumulate in many tissues and organs (Babatin *et al.,* 2008). As a result, this causes toxicities and side effects in some areas of the body. The liver is one of the tissues and organs that is most impacted (Vitins *et al.,* 2014), lung (Kuzmanova et al., 2014), kidney (Luciani *et al.,* 2009), thyroid (Hybel *et al.,* 2015), brain (Pomponio *et al.,* 2015) and ocular tissue (Turk *et al.,* 2015). Steatosis, enlarged hepatocytes, inflammation, fibrosis, and lamellar lysosomal inclusion bodies, which reflect phospholipidosis, are the hallmarks of amiodarone-induced liver damage (Lewis *et al.,* 1990). While a variety of metabolic, cellular, and immunologic changes brought on by amiodarone may be the cause of its harmful effects (Reasor and Kacew, 1996). The majority of oral amiodarone side effects, including hepatotoxicity, were caused by gradual tissue buildup and generally appeared after long-term treatment (Kowey *et al.,* 1997). Amiodarone frequently caused steatosis and liver phospholipidosis in people (Lewis *et al.,* 1989) as well as animals (Agoston *et al.,* 2003). Amiodarone's prolonged half-life causes persistent liver damage that may develop in steatosis that is similar to alcoholic liver disease (Singhal *et al.,* 2003). Decompensated liver failure caused by amiodarone-induced hepatotoxicity can eventually lead to cirrhosis, however this rarely occurs (Lewis *et al.,* 1989). Amiodarone users frequently have abnormal liver enzyme findings. Additionally, hepatomegaly and hepatitis are seen (Mattar *et al.,* 2009). Pseudo-alcoholic cirrhosis has been linked to low-dose amiodarone (Singhal et al., 2003). Amiodarone also affects the thyroid, which could negate some of its heart-healthy effects (Lombardi *et al.,* 1990). Holt *et al.* (1983) reported that amiodarone is found in a variety of tissues, including adipose tissue, the liver, the lung, and to a lesser amount, the kidneys, heart, skeletal muscle, thyroid, and brain, from where it is gradually released. Jacobs (1987) and Pollak *et al.* (1993) reported a rise in serum creatinine brought on by using amiodarone. AMD causes nephrotoxicity, which is accompanied by a significant increase in serum creatinine and blood urea nitrogen (Sakr and El-Gamal, 2016). AMD's direct cellular toxicity may occur from lysosomal phospholipase being inhibited, which causes lysosomal phospholipids to build up and cause cell death (Somani *et al.,* 1990), or as a result of a buildup of free radicals (Ruch *et al.,* 1991). Additionally, IL-6 and TNF-α indicators of inflammation are raised by AMD (Yin et al., 2019). Amiodarone produces free radicals both in vitro and in vivo, which are the primary sources of oxidative stress, according to the results of numerous research (Bouhifd *et al.,* 2013; Bell *et al.,* 2016). Reactive oxygen species (ROS), which are a symptom of oxidative stress, are produced in cells (Chen *et al.,* 2009).

Zizyphus spina christi (ZSC), also known as Christ's Thorn Jujube, Nabka, and sidr in local dialects, is one of the plants frequently used in traditional medicine to cure a variety of illnesses. It is a natural plant that thrives in warm, subtropical climates, particularly in the Middle East. ZSC belongs to the family Rhamnaceae (Youssef *et al.,* 2011). Alternative medicine frequently employs *Zizyphus* species to treat a range of illnesses, including those involving the digestive system, the liver, the urinary system, weakness, appetite loss, obesity, diabetes, bronchitis, fever, sleeplessness, pharyngitis, anemia, diarrhea, and skin infections (Basuny *et al.,* 2013). ZSC leaves extract (ZSCLE) has been shown in prior studies to contain a wide range of phytochemical components, including flavonoids, alkaloids, tannins, triterpenoids, phytosterols, saponins, and essential oils (Kadioglu *et al.,* 2016). Antioxidant, anti-inflammatory, hepatoprotective, hypoglycemic, anticancer, hypotensive, antidiarrheal, antimalarial, antiplasmodium, and immunomodulatory actions are just a few of the therapeutic properties of the genus *Zizyphus* (Dkhil *et al.,* 2018).

From this point of view, it was interesting to investigate the possible protective and anti-inflammatory effects of ZSC methanolic extract on the liver and kidney organs agonist amiodarone, which causes hepatotoxicity and nephrotoxicity in male rats.

2. Materials and Methods

2.1 Experimental animals

Twenty-four adult male albino rats; from the central animal facility of King Abdul-Aziz University, Jeddah, Saudi Arabia; were used. Animals weighing (160 – 180 g) housed at room temperature (30 ± 5 ^{o}C) in stainless steel cages, a 12/12 hour light/dark cycle, and enough ventilation and obtained complete diet pellets and water ad-libitum. Animals kept under observation for approximately two weeks before the start of the experiment to rule out any intercurrent infections.

2.2 Chemicals

Amiodarone (amiodarone hydrochloride) was imported under the license of (EBEWE Pharma Ges.m.b.H. Nfg. KG A-4866 Unterach, AUSTRIA) from El-Nahdi pharmacy (Almadinah Almonawarah, Saudi Arabia). The tablets with the brand name Cordarone 200 mg per tablet.

2.3 Plant materials and extraction

Leaves of ZSC were acquired from a local market (Almarwani for Herb) in Al-Madinah Al-Monawarah. The leaves were systematically distinguished and confirmed, where a voucher specimen number (ZIZ. 7/2020) was deposited in Department of Pharmacognosy and Pharmaceutical Chemistry, College of Pharmacy, Taibah University.

The MeOH extract of the air-dried fine powder of leaves prepared as follow: dried powder was defatted using chloroform for 72 hours and then maceration was conducted using methanol for 72 hours with intermittent shaking for methanolic extract preparation. Filtration was done and then distillation was performed to remove the solvent. The product hence obtained was reduced to a dark colored mass by distillation on rotary evaporator for further solvent elimination, then the extract was refrigerated for storage.

2.4 Dose and treatment procedure

The Amiodarone dose was 100 mg/kg body weight (Gabin *et al.,* 2020). This dose was given orally and daily for 14 days by gastric intubation. The dose of ZSC used was 200 mg/kg body weight (Mohamed *et al.,* 2017) for 14 consecutive days by gastric intubation.

2.5 Animal grouping

Animals were disaggregated into three groups, with eight rats in every group, as follows:

1. The 1st group served as a healthy control group and received normal saline 0.9% solution.
2. The 2nd group received AMD orally of a dose (100 mg/kg body weight) daily for 14 days (Gabin *et al.,* 2020).
3. The 3rd group received AMD orally of a dose (100 mg/kg body weight) daily for 14 days, then after 1 h treated with ZSC orally of a dose (200 mg/kg body weight) daily for 14 days (Mohamed *et al.,* 2017).

2.6 Ethical consideration

This study was carried out in accordance with the recommendations for the care and the use of laboratory animals. Every effort was made to reduce the quantity of used animals as well as their suffering. The research study has received the confirmation of the Ethical Committee of Taibah University, KSA (IRB Reg. No. COPTU-RIC 20210113) approved the protocol for this study.

2.7 Biochemical study

By the end of the experiment, rats were anesthetized by 500 µL of Ketamine-Xylazine intraperitoneal injection (100 and 20 mg/kg body weight, respectively), and blood was obtained from the retro-orbital venous plexus 24 hours following the most recent treatment.

Then blood was collected and left to coagulate for 40 minutes at room temperature. For biochemical analysis, sera were separated by centrifugation at 5000 rpm for 20 minutes at 20 °C and then frozen at -20 °C. The levels of urea, creatinine, albumin, ALP, AST and ALT were then measured with commercially available kits (Muslco SJ, Saudi Arabia).

Evaluation of inflammatory interleukins

Evaluation of inflammatory cytokine (interleukins) such as some inflammatory markers (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) were tested in serum using enzyme-linked immunosorbent assay (ELISA) kits, as directed by the manufacturer.

2.8 Histopathological examination

Further liver and kidney tissue sections were merged in 10% buffered formalin, washed with tap water, and dehydrated in various dilutions of 100% ethanol. Microtome sections of paraffin wax tissue with a thickness of 4 microns were created. The tissue sections were then put on glass slides and stained with hematoxylin and eosin before being examined under a microscope for histological purposes (Bancroft and Gamble, 2002).

2.9 Statistical analysis

The obtained data were analyzed by one-way ANOVA, after that; post hoc multiple comparisons LSD's test using the SPSS statistical package v22.0 for Windows (IBM, Armonk, NY, USA). *p* values less than 0.05 were regarded as statistically significant differences. The analyzed data are presented as the mean ± standard error of mean (SEM).

3. Results and discussion

3.1 The influence of ZSC on serum liver (AST, ALT, ALP, albumin) and kidney (urea and creatinine) concentration

In animals, AMD (100 mg/kg/day) caused liver damage, as demonstrated by significant increases in AST, ALT and ALP to 58.48%, 19.27% and 82 % respectively, and decrease albumin to 19.1% as compared to the normal control group. When compared to the AMD – positive control group, (ZSC) (200 mg/kg/day) significantly reduced high serum AST, ALT and ALP and albumin to 16.77%, 27.36% and 21.7 % respectively, and increases albumin to 3.56% (Table 1) and (Figures 1, 2, 3 and 4).

In animals, AMD caused kidney impairment, as demonstrated by significant increases in serum urea and creatinine to 50.56 % and 182.37 %, respectively, as compared to the normal control group. When compared to the AMD- positive control group, ZSC reduced raised serum urea by 23.3 % and reduced elevated serum creatinine by 35.16 %, respectively (Table 1) and (Figures 5 and 6).

3.2 The influence of ZSC on serum inflammatory cytokines levels

The level of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum of AMD -treated rats revealed a significant increment ($p < 0.05$) as compared with controls. However, AMD (100 mg/kg/day) resulted in the significant elevation of the level of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum to 821.06%, 187 %,

Table 1. Effects of *Zizyphus spina christi* (ZSC) on serum AST, ALT, creatinine and urea levels in amiodarone AMD -induced hepato-renal injury in rats

Groups	Hepatic function test			Renal function test		
	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)	Urea (mg/dl)	ALBU (g/dl)
Normal control	135.04 ± 0.73	40.28 ± 0.57	87.98 ± 0.42	2.78 ± 0.01	45.02 ± 0.63	3.82 ± 0.04
AMD-control (100 mg/kg, P.O)	213.95^{a} ± 2.10	88.32^{a} ± 1.06	160.18^{a} ± 1.64	7.84^{a} ± 0.07	67.75^{a} ± 0.77	3.10^{a} ± 0.16
AMD +ZSC (200 mg/kg, P.O.)	$178.1^{a,b}$ ± 1.42	$64.18^{a,b}$ ± 0.64	$125.44^{a,b}$ ± 0.68	$5.09^{a,b}$ ± 0.08	$51.96^{a,b}$ ± 0.49	$3.21^{a,b}$ ± 0.02

577.82%, 467.28%, 76 %, 147.55 % and 126.98 % respectively in comparison to the typical control group. Combined treatment with ZSC (200 mg/kg/day) significantly reduced ($p < 0.05$) the elevated level of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in serum to 83.71 %, 75.8 %, 87.46%, 82.21%, 46.4 %, 57.81 % and 52.22 %; respectively in comparison to AMD- positive control group (Tables 2 and 3) and Figure 7 (a, b, c, d, e, f and g).

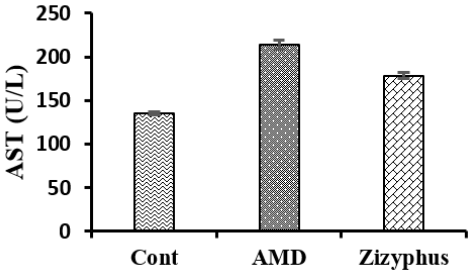

Figure 1. Effect of ZSC on AMD-treated rats' serum AST activity

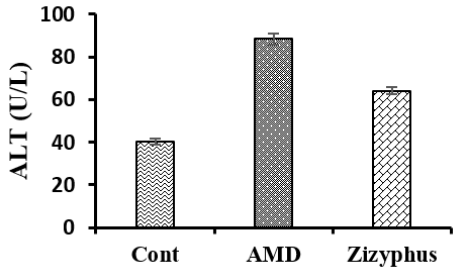

Figure 2. Effect of ZSC on AMD-treated rats' serum ALT activity

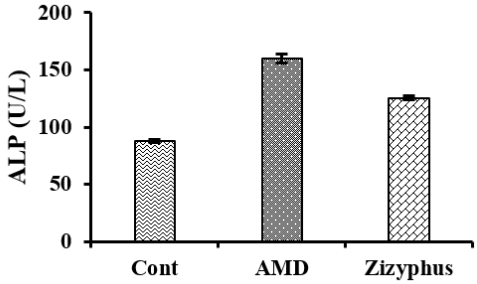

Figure 3. Effect of ZSC on AMD-treated rats' serum ALP level

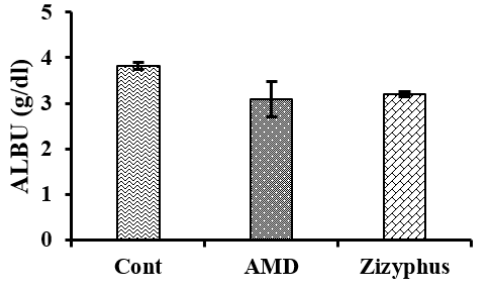

Figure 4. Effect of ZSC on AMD-treated rats' serum ALBU level

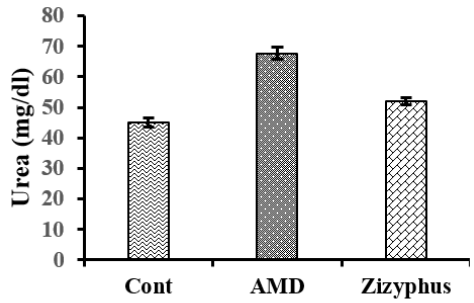

Figure 5. Effect of ZSC on AMD-treated rats' serum urea concentration

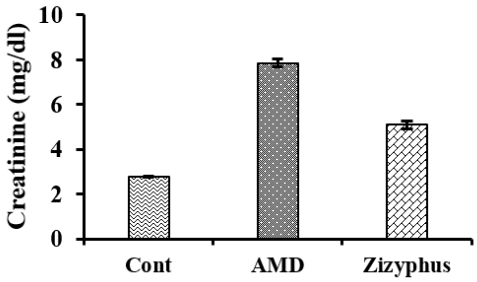

Figure 6. Effect of ZSC on AMD-treated rats' serum creatinine concentration

Table 2. Effects of *Zizyphus spina christi* (ZSC) on serum inflammatory cytokines level in amiodarone AMD -induced hepato-renal damage in rats

Groups	IL-1α (pg/mL)	IL-1β (pg/mL)	IL-4 (pg/mL)	IL-6 (pg/mL)
Normal ontrol	178.55 ± 1.66	808.24 ± 1.6	150.38 ± 4.34	1110.49 ± 17.43
AMD-control (100 mg/kg, P.O)	1644.55^{a} ± 81.82	2319.8^{a} ± 107.7	1019.3^{a} ± 34.25	6299.57^{a} ± 43.56
AMD +ZSC (200 mg/kg, P.O.)	$267.84^{a,b}$ ± 3.45	$561.16^{a,b}$ ± 26.1	$127.86^{a,b}$ ± 1.9	$1120.41^{a,b}$ ± 16.26

Table 3. Effects of *Zizyphus spina christi* (ZSC) on serum inflammatory cytokines level in amiodarone AMD -induced hepato-renal damage in rats

Groups	IL-10 (pg/mL)	IFN-γ (pg/mL)	IFN-α (pg/mL)
Normal control	656.14 ± 4.03	545.69 ± 2.60	232.95 ± 2.95
AMD-control (100 mg/kg, P.O)	1155.06[a] ± 2.67	1350.84[a] ± 10.58	528.75[a] ± 2.72
AMD + ZSC (200 mg/kg, P.O.)	619.11[a,b] ± 1.49	569.89[a,b] ± 1.25	252.62[a,b] ± 0.68

Data in all tables are offered as mean ± SEM

[a]Significantly diverse from Normal control group at $p < 0.05$ (LSD's test).

[b]Significantly diverse from AMD-positive control group at $p < 0.05$ (LSD's test).

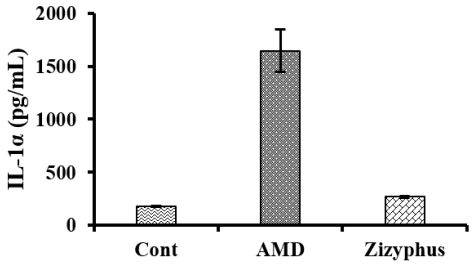

Figure 7. a) Effect of ZSC on AMD -treated rats' serum IL-1α level.

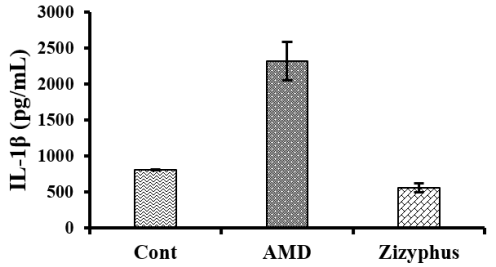

Figure 7. b) Effect of ZSC on AMD -treated rats' serum IL-1β level.

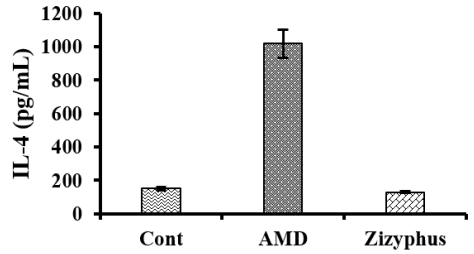

Figure 7. c) Effect of ZSC on AMD -treated rats' serum IL- 4 level.

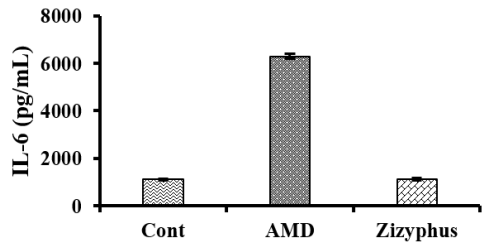

Figure 7. d) Effect of ZSC on AMD -treated rats' serum IL- 6 level.

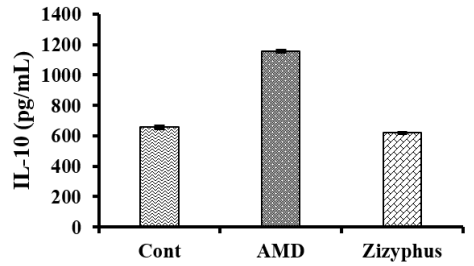

Figure 7. e) Effect of ZSC on AMD -treated rats' serum IL- 10 level.

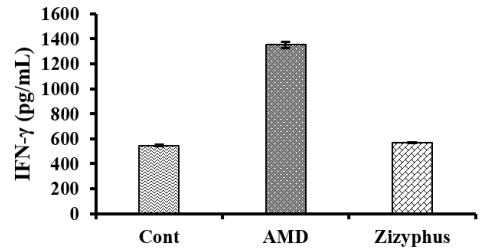

Figure 7. f) Effect of ZSC on AMD -treated rats' serum IFN-γ level.

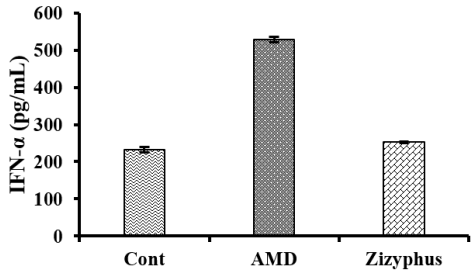

Figure 7. g) Effect of ZSC on AMD -treated rats' serum IFN-α level.

3.3 Histopathological examination of hepatic and renal tissues

The normal control rats' hepatic sections show a normal histological structure, with normal architecture, no histopathological changes were noticed in the liver of the control group. Livers of these animals shows a conventional arrangement of normal hepatocytes in cords (arrows) radiating from a regular hepatic central venule (CV). The hepatocyte cords are separated by blood sinusoids (BS) that are lined by endothelium (e). The hepatocytes (H) have acidophilic cytoplasm (C) and central vesicular nuclei (N) (Figure 8. a).

However, hepatic section from AMD-treated rat shows dilated congested central venule (CV*) and blood sinusoids (BS*). Some hepatocytes (H*) exhibit vacuolated cytoplasm (C*) and ill-defined nuclei (N*). In addition, most of the hepatocytes are disarranged (Figure 8. b). Liver of AMD group treated with ZSC (group 3) reveals a slightly preserved hepatic architecture; where the central venule (CV) has lesser congestion while the blood sinusoids (BS*) still congested. Most of the hepatocytes are preserved (H) and arranged in cords (arrows), while few hepatocytes (H*) have ill-defined nuclei (Figure 8. c).

The renal sections of the normal control rat's shows normal architecture of the renal cortex with its covering renal capsule (Cap). The cortex displays normal renal corpuscles and tubules. The cortical renal corpuscle exhibits the Bowman's capsule (BC) with conventional Bowman's space (BS) and the glomerulus (G). The cortical renal tubules reveal proximal convoluted tubules (PT) lined by acidophilic tall cuboidal epithelium with brush border and narrow lumen, distal convoluted tubules (DT) lined by smaller cuboidal epithelium with wide lumen (Figure 9. a).

Renal section from AMD-treated rats exhibits the renal cortex, beneath the detached renal capsule (Cap), with some proximal convoluted tubules (PT*) and distal convoluted tubules (DT*) having degenerated epithelial cells with vacuolated cytoplasm. Some tubules display eosinophilic hyaline cast (arrow). Also, interstitial vascular congestion (arrowhead) and glomerular vascular congestion (G*) are noticed (Figure 9. b).

For the toxic AMD group treated with ZSC reveals the renal cortex beneath the renal capsule (Cap) with few proximal (PT*) and distal convoluted tubules (DT*) that exhibit vacuolation. Numerous glomeruli (G) and many proximal (PT) and distal convoluted tubules (DT) are preserved. However, there is little interstitial vascular congestion (arrowhead) and glomerular vascular congestion (G*) (Figure 9. c).

The main goal of this study was to discover certain ZSC properties that might be beneficial in protecting rats from AMD side effects. There is substantial debate regarding amiodarone's liver damage in test animals. Amiodarone has been shown to cause liver damage in mice pre-treated with dexamethasone. This effect was caused by increased production of hazardous metabolites (Takai *et al.,* 2016). Rats exposed to amiodarone during mild inflammation produced on by lipopolysaccharide pre-treatment also showed liver damage (Lu *et al.,* 2012). However, mice given daily oral amiodarone treatment for two weeks were shown to have minimal liver damage (Zaki and Eid, 2009).

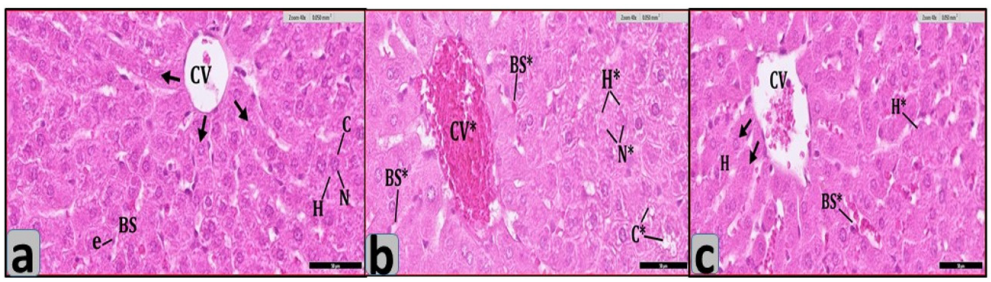

Figure 8. H&E-stained photomicrographs of liver sections of control group (a), AMD-treated group (b), AMD + ZSC co-treated group (c). All labels were explained in the main text. Scale bars = 50 µm.

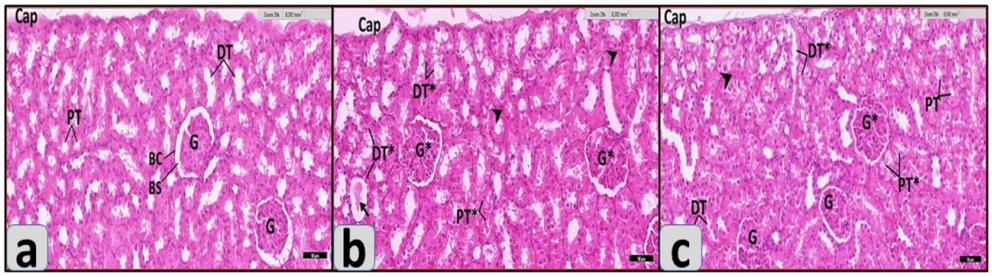

Figure 9. H&E-stained photomicrographs of kidney sections of control group (a), AMD-positive treated group (b), AMD + ZSC co-treated group (c). All labels were explained in the main text. Scale bars = 50 μm.

According to this investigation, amiodarone caused substantial liver damage as seen by noticeably raised serum ALT and AST activity, this finding agrees with Li *et al.* (2015) and Abdul-Hamid *et al.* (2018) who reported that intravenous administration of amiodarone caused elevated AST and ALT. AST is a helpful indicator of liver pathology, and the AST/ALT ratio can be used to predict cirrhosis (Anderson *et al.,* 2000). Since there is a known correlation between liver damage and an increase in the relevant liver enzymes, these values are typically utilized as indicators of hepatocellular damage (Abdel Moneim, 2016). In this regard, an increase in the activity of serum hepatocyte cytoplasmic enzymes, serum ALT and AST suggests hepatocyte damage and necrotic lesions that result in a loss of hepatocyte membrane strength and subsequent enzyme leakage into the blood as well as hepatic tissue dysfunction and disruption of the biosynthesis of these enzymes (Ramadan *et al.,* 2021). The study's findings showed that AMD causes histological changes in rat liver and kidney tissues, as shown by rises in serum AST, ALT, ALP, urea, and creatinine, as well as a fall in albumin concentration. Hepatic cell necrosis was noted in the toxic AMD group, as were liver sections; several hepatocytes had extensive vacuolation and central vein damage. The extent of amiodarone adverse effects is influenced by the total amount of medication exposure. Typically, liver damage is transient and will subside if the dose is lowered or released. It is widely known that the liver damage brought on by amiodarone is increased by the enzymes (AST) and (ALT) (Gabin *et al.,* 2020). Additionally, the outcomes of this study showed that rats' kidneys were affected histopathologically by amiodarone. Furthermore, animals treated with AMD showed a notable increase in their levels of urea and creatinine. Urea and creatinine levels in the blood are well-known indicators of acute renal damage (Bonventre *et al.,* 2010). Similar histological alterations, such as decreased glomerular filtration or reduced renal tubule reabsorption as a result of tubular epithelial cell degeneration, were seen in the kidney of rats treated with AMD in another investigation (Sakr and El-Gamal, 2013). These results were also supported by (Eid *et al.,* 2021). The results of the current study showed that oral administration of methanolic extract of ZSC to AMD-treated animals have enhanced effect agonist hepatotoxicity. It also showed a decrease of liver enzymes AST, ALT and ALP in addition urea and creatinine, furthermore, an increase in albumin concentration when compared to the AMD-positive control group, which may be due to phytochemical compound in extract of ZSC which act as antioxidant substance serve inhibition free radicals-induced lipid peroxidation and suppression AMD toxicity (Obaid, 2016). ZSC phytochemical examination revealed the presence of triterpenoidsaponins, cyclopeptide alkaloids, flavonoids, sterols, and tannins (Nawwar *et al.,* 1984), Consequently, this substance has antioxidant properties that scavenge free radicals (Basuny *et al.,* 2013), This result agree with Yossef and co-workers (2011) who reported ZSC has a protective impact, antioxidant activity that is an agonist of oxidative stress and hepatic toxicity, and it lowers blood levels of liver enzymes while restoring normal levels of endogenous antioxidants (Youssef *et al.,* 2011).

Inflammation is related to greater levels of (ROS). Pro-inflammatory cytokines, for example TNF-α and IL-1β, cause macrophages and neutrophils to produce (ROS) (Lei *et al.,* 2014). Pro-inflammatory cytokine concentrations are a sign of continuing inflammation (Jekarl *et al.,* 2013). It has been demonstrated that blood levels of IL-6 are elevated in both acute and chronic liver disease (Streetz et al., 2003). As per the present study, the level of inflammatory cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) rise significantly in the serum of AMD-treated animals. These results are in harmony with prior findings by Eid and co-workers (2021) who demonstrated that the AMD-treated animals had an increased expression of serum IL-6. Interestingly, the co-administration of ZSC and AMD, significantly lowered the levels of these cytokines (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) when compared to AMD-positive control rats. This conclusion is completely compatible with previous findings (Huang *et al.,* 2017; Alajmi *et al.,* 2019). In this context, and in support to biochemical and histological findings, the supplementation of ZSC prevented AMD-induced inflammation as demonstrated by the decreased levels of (IL-1α, IL-1β, IL-4, IL-6, IL-10, IFN-γ and IFN-α) in blood serum. These findings showed that ZSC mediated suppression of inflammation plays a central role in their hepato-renal protective effects.

4. Conclusion

This study concludes that ZSC defends against AMD induced hepatic and renal injury by rising biochemical parameters such as AST, ALT, ALP, urea, creatinine and decreasing ALBU levels, as well as preventing soft tissue destruction, and inflammation in relation to AMD treatment. The obtained results are in line with the improvement of histological abnormalities in the liver and kidney organs. Therefore, ZSC could be used as an adjuvant remedy to protect against amiodarone induced toxicity and consider anti-inflammatory mediators.

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