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Intermittent Fasting Attenuates Adriamycin-induced Hepatoxicity in Rats: Possible Role for Nrf2/HO-1 and LC3/Sirt1 Pathways

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Abstract

Background: The current study was designed to examine, whether intermittent fasting can ameliorate the liver damage induced by adriamycin (ADR) in rats, as well as its possible underlying mechanisms.
Methods: Forty male Sprague Dawley rats were allocated into 4 equal groups, control, fasting, ADR and ADR+ Fasting groups. At the end of the experiment (eight weeks after ADR administration), blood samples were collected for the measurement of ALT, AST, and albumin, and liver tissues were harvested for biochemical analyses of oxidative stress markers (AMD, GSH and Catalase). Real-time PCR was performed for NRF2 and HO-1, as well as histopathological examination and immunostaining for caspase-3, LC3, and Sirt-1 expression.
Results: Administration of ADR caused significant elevation in liver enzymes (ALT, AST), lipid peroxidation (MDA), histopathological damage score and caspase-3 in liver tissues ($p < 0.05$) with a notable decrease in GSH, catalase, Nrf2, HO-1, LC3, and Sirt-1 genes expression ($p < 0.05$). Conversely, the application of intermittent fasting (IF) to ADR-treated rats caused significant attenuation of the raised liver enzymes (ALT, AST), lipid peroxidation (MDA), histopathological damage score and caspase-3 in liver tissues and significant improvement in the attenuated GSH, catalase, Nrf2, HO-1, Lc3 and Sirt-1 gene expression ($p < 0.05$).
Conclusion: Intermittent fasting could potentially offer protection against ADR-induced hepatotoxicity in rats by reducing oxidative stress and apoptosis and modifying the expression of Nrf2/HO-1, LC3, and Sirt-1.

Keywords: Adriamycin, Hepatoxicity, Intermittent Fasting, LC3, Nrf2, Oxidative Stress, Sirt-1.

Introduction

One of the most potent chemotherapeutics against cancer is Adriamycin (ADR), an anthracycline antibiotic with a broad-spectrum action (1). It can be used alone or in combination with other drugs to treat a range of solid and hematological malignancies, including breast cancer (2). Adriamycin (ADR) is associated with considerable liver damage and other organ toxicities (3). This may limit its clinical relevance in cancer treatment even though it is a therapeutically effective agent. It also

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inhibits topoisomerase type II, reduces the antioxidant defense system, interferes with cell division, induces oxidative stress, and ultimately leads to cellular necrosis or death in the cells (4). Although various factors such as inflammation, apoptosis, DNA damage, impaired calcium metabolism, increased free radical production, and downregulation of antioxidant genes may directly contribute to deterioration of organ function, the precise mechanisms underlying ADR-mediated multiple organ toxicity require further clarifications (3). The primary cause of ADR-induced adverse effects in humans and animals is thought to be a significant increase in reactive oxygen species (ROS) whitin cells, which eventually leads to cellular damage and apoptosis (5). Moreover, oxidative stress has been shown to be closely associated with ADR-induced toxicity in various organs (6). Consequently, combining ADR with antioxidants (3), like natural flavonoids, may help reduce or prevent. ADR-induced side effects.

Intermittent fasting (IF) has been shown to extend life expectancy and enhance health in mammals (7). Several patterns of IF have been thoroughly examined in human patients and animal models, and they have been shown to treat a wide range of health conditions, including obesity, impaired glucose tolerance, dyslipidemia, hypertension, reproductive problems, liver impairment, and neurodegenerative diseases (8). The mechanisms that underlie IF's cytoprotective effects include autophagy, mitochondrial biogenesis, protein quality control, DNA repair, antioxidant defenses, and the suppression of inflammatory responses (9). Through the activation of anti-stress responses such as the nuclear factor erythroid 2-related factor 2 (Nrf-2) and the mammalian target of rapamycin (mTOR) pathways, intermittent fasting (IF) also protects against inflammation and oxidative damage (10). In several organs and tissues, such as the brain, kidney, and colon, it has been demonstrated that the Nrf2/heme oxygenase (HO)-1 signaling pathway is crucial in regulating oxidative stress and inflammatory activity (11). HO-1, glutathione (GSH) and superoxide dismutase (SOD) are examples of downstream proteins whose transcription, modification, or expression are regulated by Nrf2 during dietary energy intake is restriction (12). Also, it has been demonstrated that IF upregulates the cytoprotective gene (Sirt-1) by switching the energy source from glucose to ketone bodies. To effectively detoxify ROS, this causes the activation of Mn-SOD, catalase, and glutathione as antioxidant mechanisms, as well as the Sirt-1 downstream transcription factors PGC-1 and Nrf2 (13). In addition, Sirt-1 inhibited the secretion of pro-inflammatory markers such as TNF, IL-1, and IL-6, as well as NF-kB, which is involved in NLRP3 inflammasome expression and the activation of apoptotic caspase-3, thereby inducing apoptosis (14). Recently, it has been demonstrated that IF ameliorates age-related kidney morphological changes through the activation of autophagy, as indicated by LC3 expression (15). For the maintenance of energy balance and cellular homeostasis, as well as cell and tissue remodeling, quality control, and defense against external stressors and pathogens, IF's activation of liver autophagy is essential. Pathways and molecular mechanisms, including energy metabolism, reactive oxygen species (ROS) handling, and the cellular stress response system, influence liver autophagy, which can protect hepatocytes from genetic and environmental insults (16). In this study, we hypothesized that IF could attenuate the ADR-induced liver damage through the regulation of Sirt-1, autophagy, oxidative stress, inflammation, and apoptosis. The current work aimed to explore the preventive effects of IF on liver functions and morphology in rats with ADR-induced hepatoxicity, as well as the potential involvement of cytoprotective (Sirt-1), autophagic (LC3), apoptotic (caspase-3), and antioxidant (Nrf2\HO-1) pathways in mediating these effects.

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Materials and Methods

Animals and drugs

Forty male Sprague-Dawely rats (weighing ~200-250 g, aged 4-6 months) were purchased and housed at the animal house of medical experimental research center (MERC), faculty of medicine, Mansoura university, and acclimatized for 1 week before the experiments. The rats were housed in room with controlled temperature (22 ± 2 °C), humidity (65%) and a 12-h dark/light cycle. Rats were fed a standard laboratory chow and had free access to tap water.

Adricin (doxorubicin HCL) in the form of vial (50 mg/ 25 ml) manufactured by Hikma Specialized Pharmaceuticals, was used in this study.

Experimental groups

After the acclimation period, there were four equal groups of rats randomly selected (10 rats in each); as the following:

a) Control group: normal rat fed standard chow without intermittent fasting (IF).

b) Fasting group: normal rats that were exposed to IF program of alternate periods of 24 hrs of fasting and feeding for 8 weeks (17).

c) ADR group: rats received two intravenous (i.v.) injections of ADR (4 mg/Kg dissolved in 0.5 ml saline) in the tail vein at 14-day interval without IF (18).

d) ADR + Fasting group: as ADR group, but rats were exposed to the same IF program of fasting group for 8 weeks.

Sample collection and analysis

Each rat was anesthetized with a high dose of sodium thiopental (120 mg/kg) intraperitoneally (i.p.) at the end of the experiment (8 weeks after ADR doses). The blood samples collected via cardiac puncture, centrifuged, and the serum was stored at -20 °C for biochemical analyses of liver functions. The animal's abdomen was then quickly opened, and the liver was removed and dissected. The separated livers were divided into two portions: one was fixed in 10% formaldehyde for histological and immunohistochemical investigations, while the other was stored at -80 °C for real-time PCR gene expression and biochemical assessments of oxidative stress markers.

Serum albumin was measured colorimetrically using kits obtained from Diamond Diagnostics (24 El Montazah St., Heliopolis, Cairo, Egypt), while serum alanine transaminase (ALT) and aspartate aminotransferase (AST) were evaluated using reagent kits obtained from Spinreact (Spinreact, S.A./S.A.U., Ctra.Santa Coloma, 7 E-17176 Sant Esteve De Bas (Gi) Spain).

Biochemical assay of oxidative stress markers

Cold phosphate-buffered saline (pH 7.4, 50 mM) was used to homogenize approximately 100 mg of the liver tissues. Colorimetric kits from Bio-Diagnostics (Giza, Egypt) were used to assess reduced glutathione (GSH), malondialdehyde (MDA), and catalase enzyme (CAT) in the tissue homogenates, following the manufacturer's instructions.

Real-time RT-PCR Analysis of Nrf2, HO-1 mRNA expression

Nuclear erythroid-related factor 2 (Nrf2) and heme oxygenase-1 (HO-1) mRNA levels were quantified using real-time PCR. Following the manufacturer's instructions, commercial RNA extraction kits were used to isolate total RNA from liver tissue samples. RNA purity was assessed by ethidium bromide staining, agarose gel electrophoresis, and spectrophotometric measurement. cDNA was made from one microgram of total RNA in a final volume of 25 µL. All PCR reaction methods and gene expression estimations were detailed in a recent study (19). Primer sequences and their corresponding accession numbers are listed in Table 1.

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Table 1. Primer sequence and accession numbers of tested genes.

Gene	Sense	Anti-sense	Accession #
Nrf2	5′ATT GCTGTCCATCTCTGTCAG-3′	5′-GCTATTTTCCATTCCCGAGTTAC-3′	**NM_001399173.1**
HO-1	5′TGCTTG TTTCGCTCTATCTCC-3′	5′-CTTTCAGAAGGGTCAGGTGTC-3′	**NM_012580.2**

Histopathological examination

Hematoxylin and eosin staining was performed on 5-µm thick slices of formalin-fixed liver tissue processed into paraffin blocks. The sections were photographed (Optika microscope software, Italy) and examined under a light microscope. Each rat's liver tissue was prepared independently, and histopathological alterations were assessed and scored. The histological evaluation (histoscore) considered 10 non-overlapping random areas at ×10 magnification, with scoring criteria defined as 0 (absent), 1 (mild), 2 (moderate), and 3 (severe) (20). Histopathological score criteria included hemorrhagic regions, inflammatory foci, degenerated hepatocyte cords, sinusoidal dilatation, and hepatocyte vacuolization.

Immunohistopathological examination for caspase-3, LC3 and Sirt-1

The tissue sections were treated with primary antibodies: goat polyclonal anti-Sirt-1 (1:200, Santa Cruz, sc-19857, Santa Cruz, CA, USA); active caspase-3 rabbit polyclonal (1:1000; catalog no. GB11532, Servicebio, Wuhan, China); and LC3 rabbit polyclonal (1:1200; catalog no. GB13431, Servicebio, Wuhan, China). Following this, the sections were incubated for 15 minutes with UltraVision One HRP Polymer. Substrate/Chromogen Solution (Reagent B1) was prepared according to the number of slides and combined with DAB Buffer Solution (Reagent B2) in a 1:1 ratio. Typically, 200 µL of the mixed substrate solution was applied to each tissue slide. The degree of DAB expression of Sirt-1, LC3, and caspase-3 was evaluated in 10 high-power fields (HPF) using ImageJ software, and the average value was calculated.

Statistical analysis

The data were processed and analyzed, and graphs were generated using GraphPad Prism software, version 6. One-way ANOVA with post-hoc Tukey's test was performed to determine statistical significance among different groups. A p-value < 0.05 was considered statistically significant.

Results

Effects of fasting on liver functions in ADR-induced hepatoxicity

Serum albumin levels were significantly decreased ($p < 0.01$), while serum ALT and AST levels were significantly elevated ($p < 0.001$) in the ADR group when compared to the control and fasting groups. However, in the ADR+Fasting group, serum albumin was significantly increased ($p < 0.05$), and serum ALT and AST were significantly decreased ($p < 0.01$) compared to the ADR group (Fig. 1).

Effects of fasting on oxidative stress markers in ADR -induced hepatoxicity

The ADR group showed a significant increase in liver MDA concentration ($p < 0.001$) with a significant decrease in liver GSH and CAT ($p < 0.001$) compared to control and fasting groups. Conversely, ADR+Fasting group showed a significant decrease in liver MDA concentration ($p < 0.001$) and a significant increase in liver GSH and CAT ($p < 0.05$) compared to the ADR group. Moreover, the fasting group demonstrated a significant increase in GSH compared to control group ($p < 0.05$) (Fig. 2).

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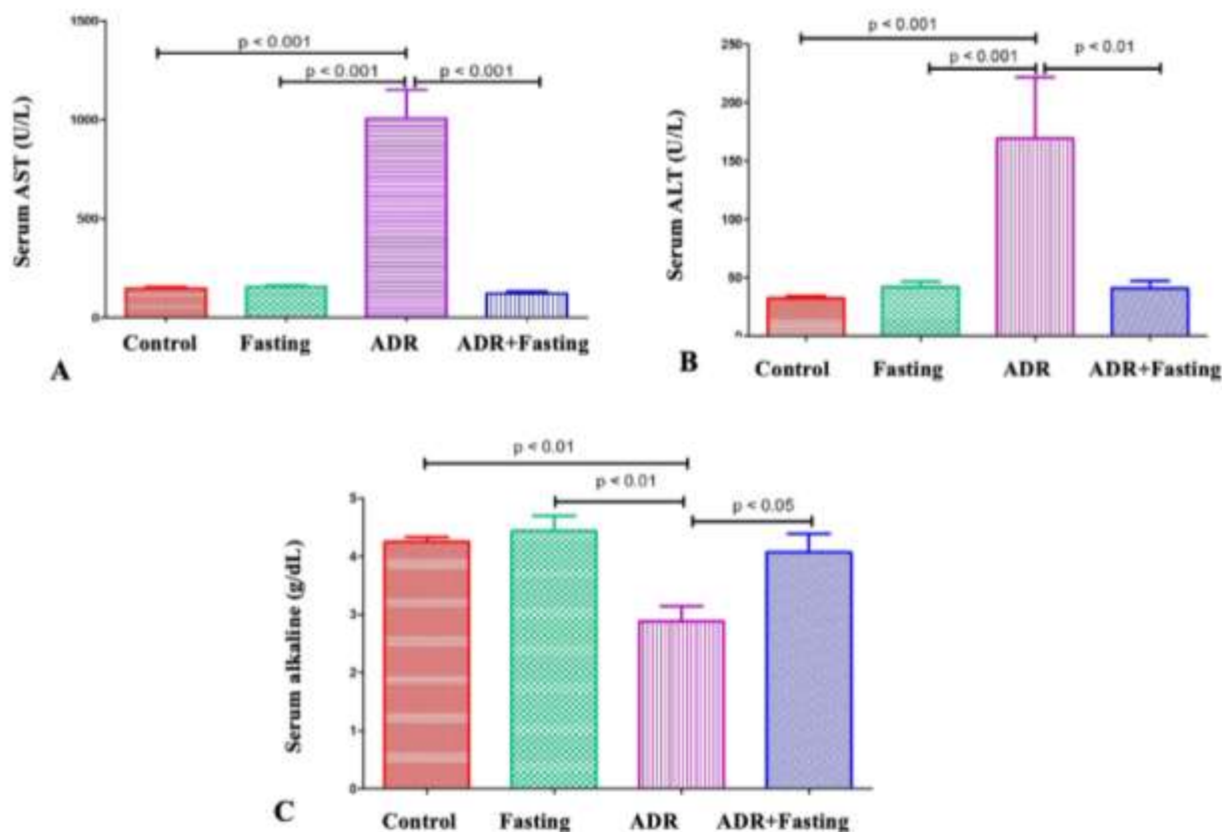

Fig. 1. Effects of intermittent fasting (IF) on liver functions. A= serum level of AST, B= serum level of ALT and C= serum albumin. ADR= adriamycin, AST= asparatate transaminase, ALT= alanine transaminase.

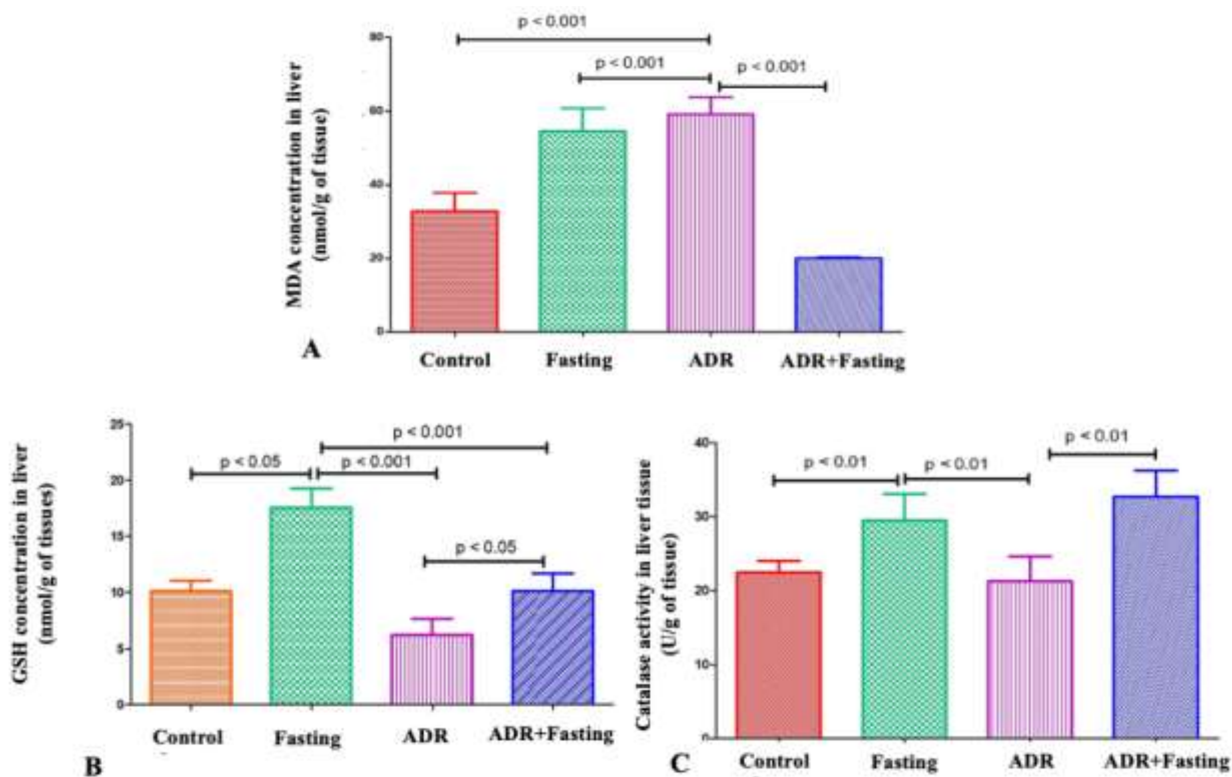

Fig. 2. Effects of intermittent fasting (IF) on oxidative stress markers in liver tissues. A= tissue level of MDA, B= tissue level of GSH and C= tissue level of catalase. ADR= adriamycin, MDA, malondialdehyde, and GSH = reduced glutathione.

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Effects of fasting on Nrf2 and HO-1 gene expression in ADR -induced hepatoxicity

ADR group showed a significant decrease in the expression of Nrf2 and HO-1 at mRNA levels in liver tissues compared to control and fasting groups ($p < 0.001$, $p < 0.01$ respectively). On the other hand, ADR+Fasting group exhibited a significant increase in liver Nrf2 and HO-1 ($p < 0.001$) compared to ADR group (Fig. 3).

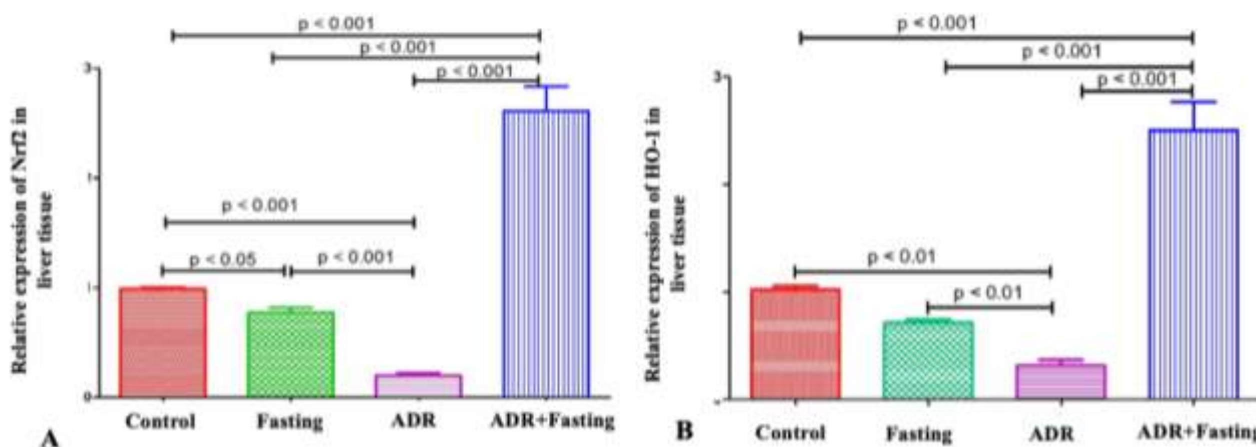

Fig. 3. Effects of intermittent fasting (IF) on gene expression in liver tissues. A= tissue level of Nrf2 and B= tissue level of HO-1. ADR= adriamycin, Nrf2= nuclear erythroid- related factor 2, HO-1= heme oxygenase-1.

Effects of fasting on histopathological damage in ADR-induced hepaotxicity

In contrast to the fasting and control groups, the ADR group showed marked histological disturbances in liver tissue ($p < 0.001$). Conversely, the ADR + fasting group exhibited significantly fewer histological alterations compared to the ADR group ($p < 0.001$) (Table 2). Photomicrographs from the control and fasting groups demonstrated regular hepatocyte cords, close arranged hepatocytes, intact sinusoidal structure, and normal liver histology (Fig. 4A & 4B, respectively). Photomicrographs obtained from the ADR group revealed sinusoidal dilatation, hepatocyte vacuolization, inflammatory foci, disrupted hepatocyte cords, and hemorrhagic areas (Fig. 4C & 4D). However, the ADR + fasting group showed amelioration of these histological changes compared to the ADR group (Figs. 4E & 4F).

Table 2. Effects of intermittent fasting on histopathological damage score in ADR-hepatoxicity.

	Control group	*Fasting group*	*ADR group*	*ADR+ Fasting group*	*P value (KW)*
Sinusoid dilatation	0.10 (0.00-0.20)	0.20 (0.00-0.30)	2.00 (1.50-2.60)*#	0.90 (0.50-1.40) *#$	< 0.001
Hepatocyte vacuolization	0.20 (0.00-0.40)	0.20 (0.10-0.40)	2.20 (1.90-2.40) *#	0.90 (0.4-1.40) $	< 0.001
Foci of inflammation	0.00 (0.00-0.10)	0.10 (0.00-0.20)	2.30 (1.80-2.70) *#	0.70 (0.5-1.30) *#$	< 0.001
Degenerated hepatocyte cords	0.10 (0.00-0.30)	0.20 (0.10-0.40)	2.50 (1.80-2.60) *#	0.80 (0.50-1.40) *#$	< 0.001
Hemorrhagic areas	0.00 (0.00-0.10)	0.10 (0.00-0.10)	2.00 (1.00-2.50) *#	0.60 (0.50-0.90) *#$	< 0.001

Data are expressed as median (min–max) * significant vs control group, #significant vs fasting group and $ significant vs ADR group. KW= Kruskal Wallis.

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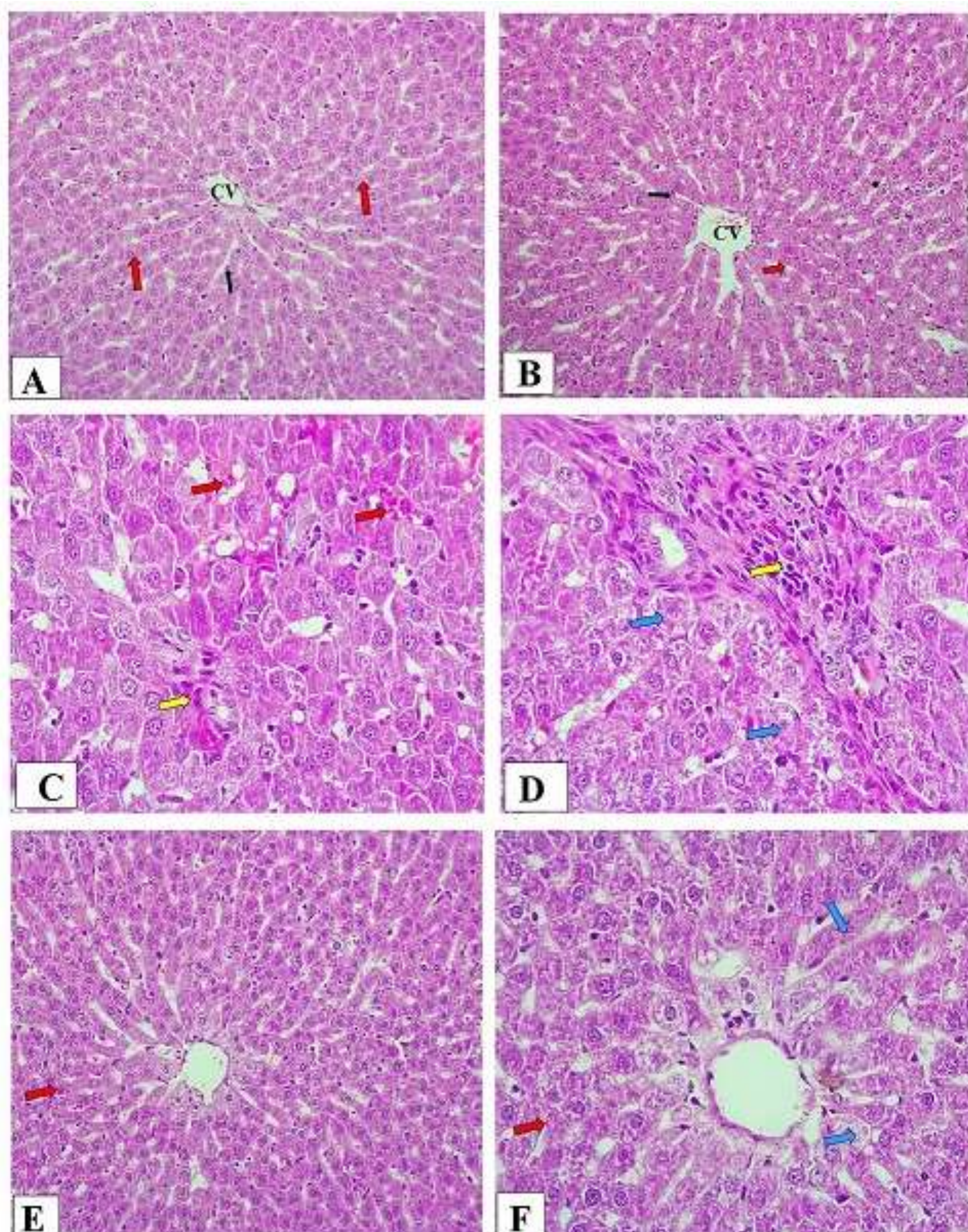

Fig. 4. Effects of intermittent fasting (IF) on histopathological changes in ADR-induced liver tissue. Photomicrographs of liver specimens obtained from control group (A H&E, 200x) and Fasting group (B, H&E, 200x) show normal histological appearance, CV= central vein, regular heptocyte cords (red arrows), narrow blood sinousoids with endotheila linning cells (black arrows). The photomicrographs obtained from ADR group (C, D, H&E, 400x) shows sinusoidal dilatation, hemorrhagic areas (red arrows), hepatocyte vacuolızation (blue arrows) and foci of inflammation (yellow arrow). Histopathological changes were significantly reduced in the ADR + fasting group (E, H&E, 200x and F, H&E, 400x).

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Effects of fasting on the expression of apoptotic marker (caspase 3) in ADR-induced hepatoxicity

Compared to the control and fasting groups, the ADR group showed a significant increase in caspase-3 expression ($p < 0.001$). This expression was significantly reduced in the ADR+ Fasting group compared to the ADR group ($p < 0.001$). The difference between the fasting and control groups is also statistically significant ($p < 0.05$) (Fig. 5A). Liver samples displayed brown cytoplasmic staining for caspase-3, which was mild in the fasting and control groups (Figs. 5B and 5C), strongly expressed in the ADR group (Fig. 5D), and reduced in the ADR + Fasting group (Fig. 5E).

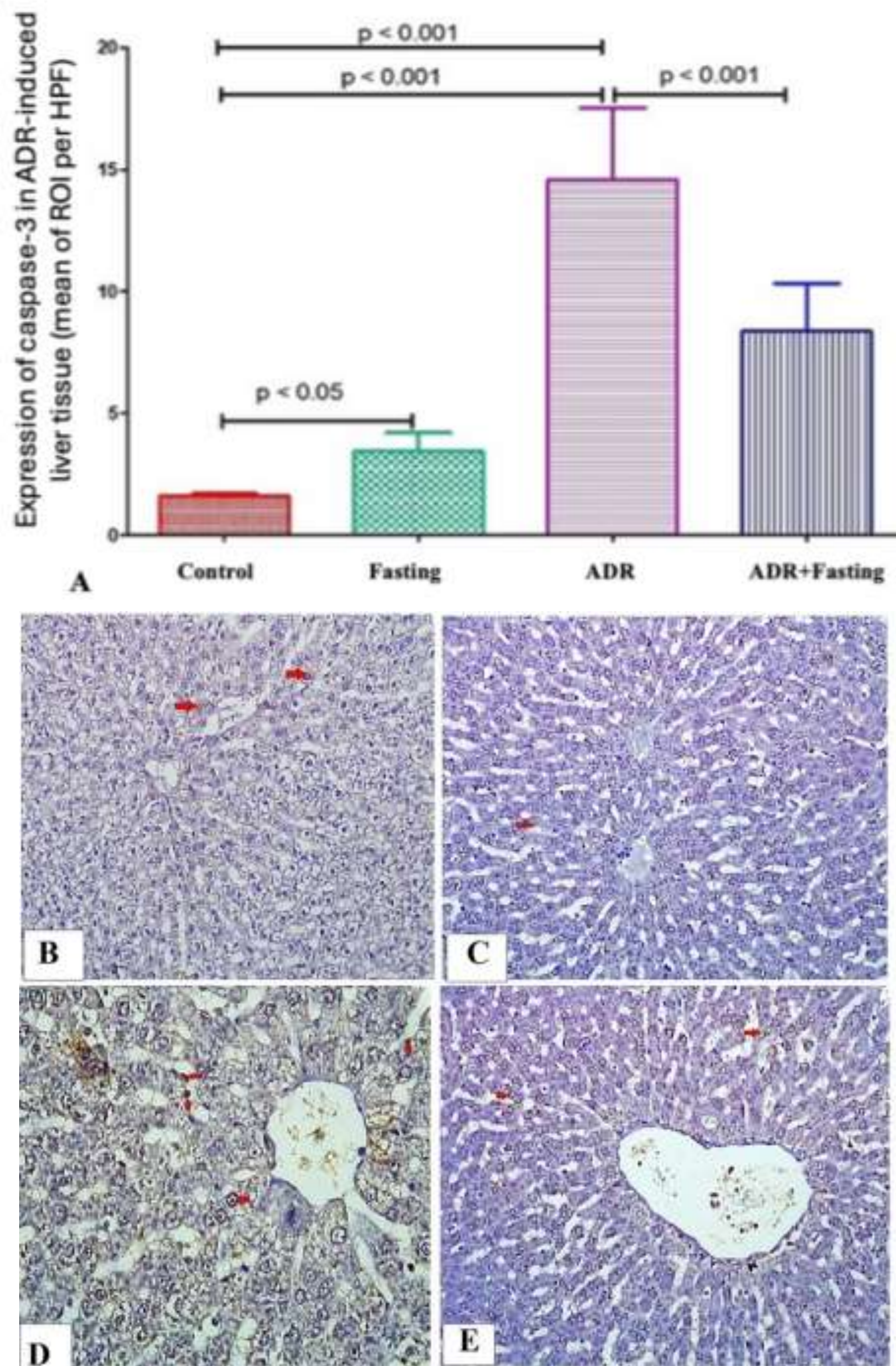

Fig. 5. Effects of intermittent fasting (IF) on expression of caspase-3 in ADR-induced liver tissue. A= score of caspase-3 expression from different groups. Photomicrographs of liver specimens showing few brown cytoplasmic staining (red arrows) for caspase-3 in liver specimens obtained from control group (B, 200x) and Fasting group (C, 200x), marked bown cytoplasmic staining (red arrows) in liver specimens obtained from ADR group (D, 400x) and few brown cytoplasmic staining (red arrows) for caspase-3 in liver specimens obtained from ADR + fasting group (E, 200x). ADR= adriamycin.

Effects of fasting on the expression of autophagy marker (LC3) in ADR -induced hepatoxicity

LC3 expression was significantly decreased in the ADR group compared to the control and fasting groups ($p < 0.05$). In contrast, the ADR+Fasting group showed a significant increase in LC3 expression compared to the ADR group ($p < 0.01$) (Fig.6A). Liver specimens displayed brown cytoplasmic

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staining for LC3, which was markedly reduced in the ADR group (Fig. 6D) compared to the control group (Fig. 6B) and fasting groups (Fig. 6C). Conversely, the ADR+Fasting group exhibited a marked increase in LC3 expression compared to the ADR group (Fig. 6E).

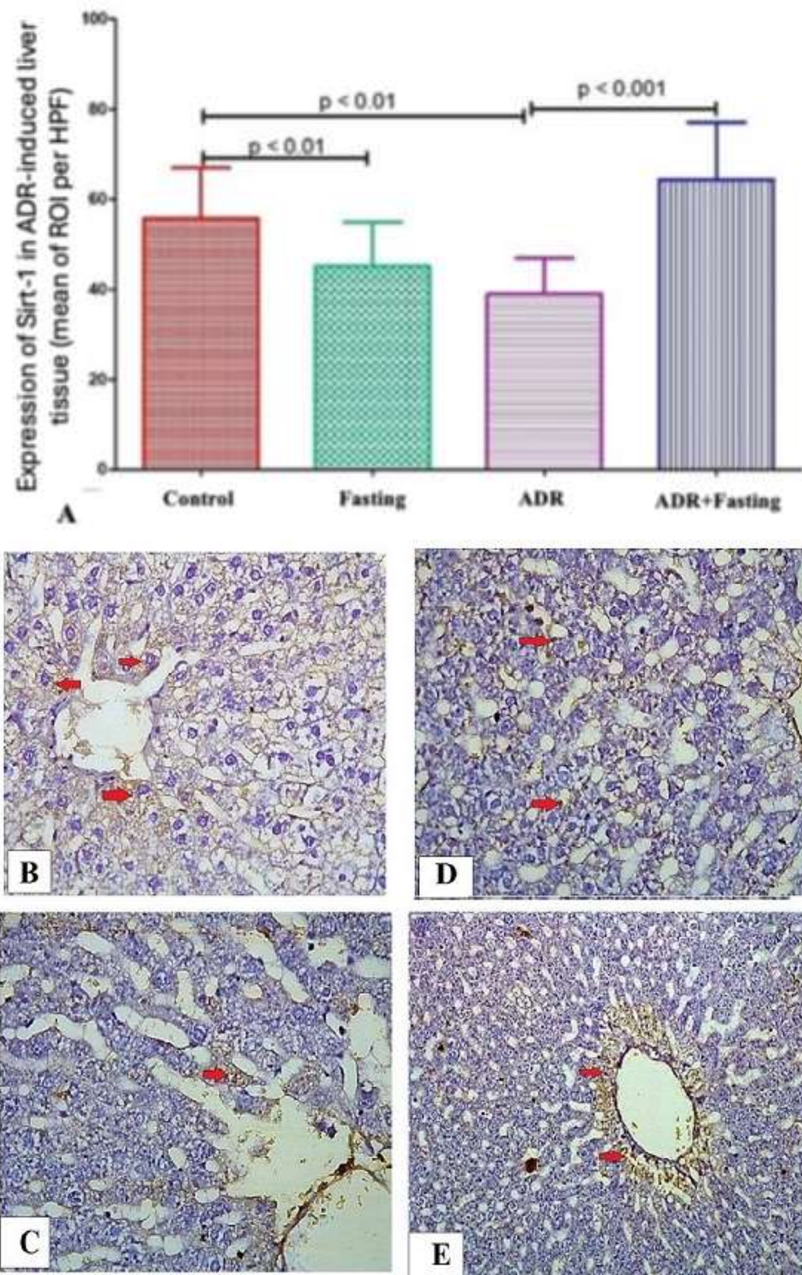

Fig. 6. Effects of intermittent fasting (IF) on expression of LC3 in ADR-induced liver tissue. A= score of Lc3 expression from different groups. Photomicrographs of liver specimens showing marked brown cytoplasmic staining (red arrows) for lc3 in liver specimens obtained from control group (B, 400x) and Fasting group (C, 400x), moderate bown cytoplasmic staining (red arrows) in liver specimens obtained from ADR group (D, 400x) and moderate brown cytoplasmic staining (red arrows) for lc3 in liver specimens obtained from ADR + fasting group (E, 200x). ADR= adriamycin.

Effects of fasting on the expression of (Sirt-1) (cytoprotective gene marker) in ADR-induced hepatoxicity

Sirt-1 expression in the ADR group was significantly lower than in the control and fasting groups ($p<0.01$). However, its expression was significantly increased in the ADR+Fasting group compared to the ADR group ($p < 0.001$). Furthermore, no statistically significant difference was observed between the fasting and control groups (Fig. 7A). Liver

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samples displayed brown cytoplasmic staining for Sirt-1, which was prominent in the fasting and control groups (Figs. 7B & 7C), weakly expressed in the ADR group (Fig. 7D), and prominent again in the ADR+Fasting group (Fig. 7E).

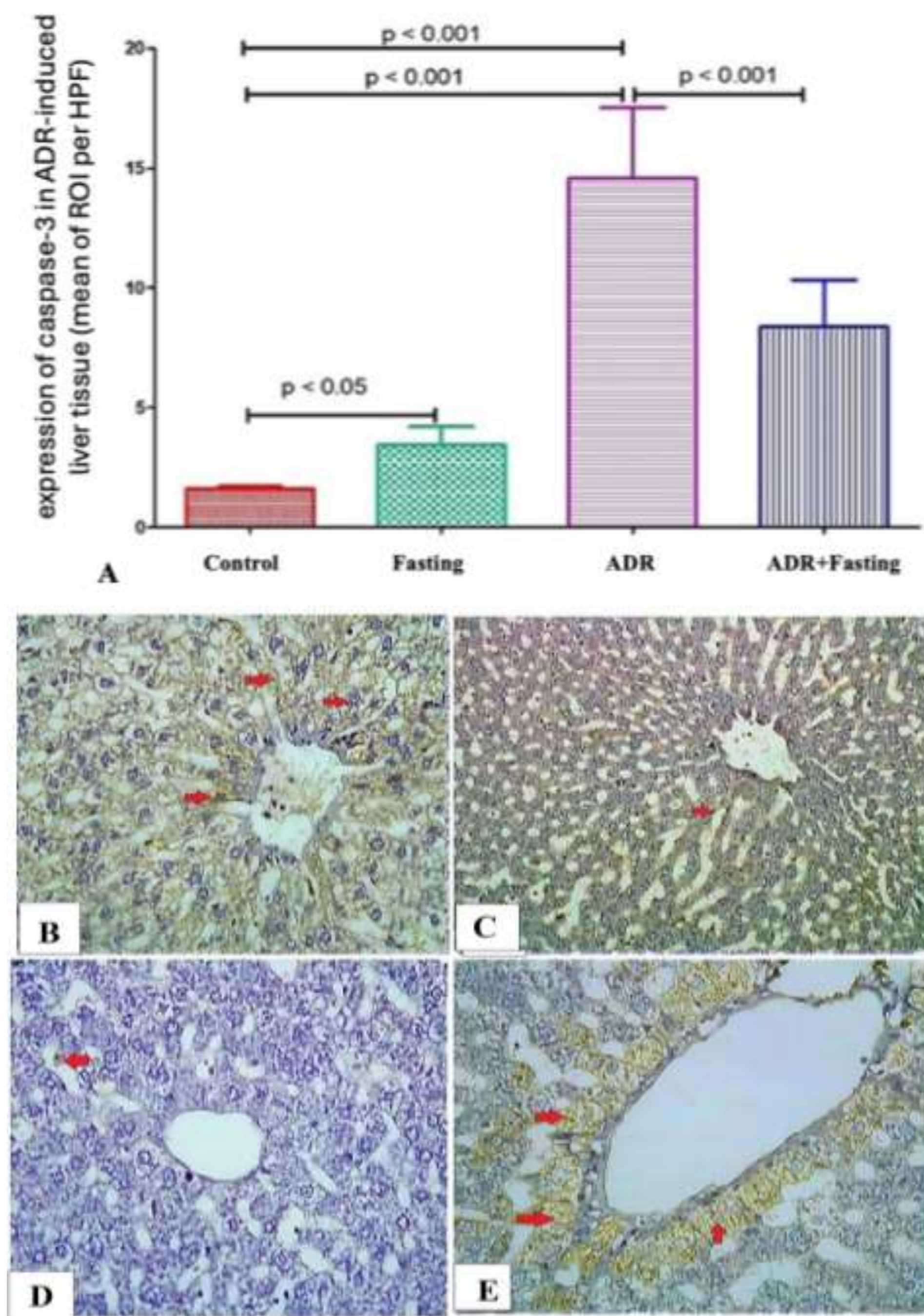

Fig. 7. Effects of intermittent fasting (IF) on expression of Sirt-1 in ADR-induced liver tissue. A= score of Sirt-1 expression from different groups. Photomicrographs of liver specimens showing marked brown cytoplasmic staining (red arrows) for Sirt-1 in liver specimens obtained from control group (B, 400x) and Fasting group (C, 400x), moderate bown cytoplasmic staining (red arrows) in liver specimens obtained from ADR group (D, 400x) and moderate brown cytoplasmic staining (red arrows) for Sirt-1 in liver specimens obtained from ADR + fasting group (E, 200x). ADR= adriamycin.

Discussion

Adriamycin-induced hepatoxicity (ADR) is a well-known cytotoxic anthracycline chemotherapeutic agent used in cancer treatment. A common side effect of ADR, which poses significant challenges during cancer therapy, is hepatotoxicity (21). The primary mechanisms underlying ADR-

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induced hepatotoxicity include apoptosis, mitochondrial dysfunction, DNA damage, and free radical damage (22). Additionally, previous studies have shown that ADR-induced cytotoxicity is strongly influenced by endoplasmic reticulum (ER) stress-mediated apoptosis (23). As interest in complementary approaches to mitigate chemotherapy-induced hepatotoxicity grows, the aim of this study was to assess the potential protective effects of intermittent fasting (IF) against ADR-induced hepatotoxicity, focusing on oxidative stress, autophagy, and apoptosis. The findings of the present study demonstrated that ADR significantly elevated serum ALT and AST levels while significantly reducing serum albumin, consistent with the results reported by Zobeydi et al. (24). Conversely, serum ALT and AST activities decreased toward normal levels in the ADR+ Fasting group. This reduction in liver enzymes with IF may reflect decreased hepatocyte damage (16) and suggests that IF could exert cytoprotective effects against ADR-induced hepatotoxicity.

Hepatic sinusoidal enlargement, hepatocyte vacuolization, sinusoid dilatation, hepatocyte cord degeneration, cellular edema, focal necrosis, irregular hepatic trabeculae, biliary duct proliferation, parenchymal necrosis, intercellular space enlargement, and lymphocyte infiltration are among the liver tissue alterations induced by ADR that have been documented in previous studies (20,21). These results demonstrate the hepatoprotective effects of IF against ADR-induced hepatotoxicity and corroborate previous research showing that fasting prevented renal and hepatic impairments (25). Additionally, Al-Kazimi et al. reported that the livers of diabetic mice appeared similar to those of normal mice and had a preserved histological appearance following a 17-hour fast. Their findings further indicate that fasting offers protection against the hepatotoxicity that diabetes induces (26).

Oxidative stress, mostly caused by ROS generation in the hepatic tissue, is the primary molecular mechanism associated with ADR-induced liver injury (27). Our study shows that rats exposed to ADR exhibited large increases in lipid peroxidation (MDA) and reduced in anti-oxidative indicators (GSH and CAT). In contrast, IF dramatically reduced MDA while increasing CAT and GSH in the ADR+ Fasting group's liver tissues, indicating a strong reduction in ADR-induced oxidative stress. These findings are in line with previous studies that demonstrated that intermittent fasting lowers oxidative stress by boosting the expression of antioxidant enzymes like catalase and superoxide dismutase and by decreasing the generation of reactive oxygen species (28). Additionally, Elsaid et al. reported that in ADR-induced nephrotoxicity, IF significantly reduced MDA while also significantly increasing CAT and GSH (29).

In reaction to oxidative stress, nuclear erythroid- related factor 2 (Nrf2) can control a variety of antioxidant proteins, such as heme oxygenase (HO)-1, and glutathione peroxidases (30). According to our data, these antioxidative parameters were significantly decreased, which is in line with earlier research showing that injecting ADR lowers NRF2, HO-1, and SOD (31). In the liver tissues of rats treated with ADR, IF markedly elevated Nrf2/HO1, suggesting that this pathway may be involved in IF's hepatoprotective effects against ADR-induced hepatotoxicity. Previous study has shown that intermittent fasting increases the expression of genes that regulate oxidative stress, such as Nrf2/HO1, which is necessary for the activation of antioxidant enzymes, which is in line with our findings (29).

According to the current study, the liver specimens from the ADR group showed apoptotic cell death as a result of the sections of animals treated with ADR exhibiting extensive positive immunoreactivity for caspase-3. This result aligns with previously published investigations (32). However, the current investigation demonstrated that IF significantly reduced caspase-3 expression and apoptotic cell death. Similar to previous researchers who discovered that IF may reduce the effects of apoptosis and restore the oxidative state, hence reducing ADR-induced cardiotoxicity, these data suggest that IF has an anti-apoptotic

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function (26). Additionally, a previous study found that in ADR-induced nephrotoxicity, IF significantly reduced caspase-3 expression and apoptotic cell death (29).

The activation of liver autophagy by IF is essential for energy balance and cell homeostasis, as well as cell and tissue remodeling, quality control, and defense against infections and external harm. Hepatic autophagy can be protected from genetic and environmental effects by a variety of pathways and molecular systems, including energy regulation, oxygen-free radical metabolism, and the cellular stress response system. These pathways reduce the expression of genes associated with aging and prevent the growth of liver tumors by enhancing the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor α (33). NAFLD, viral hepatitis, liver fibrosis, hepatocellular carcinoma, and drug-induced liver injury are among the liver illnesses that IF may be able to ameliorate by triggering liver autophagy (34). Our investigation discovered that the ADR group's liver tissues had significantly lower levels of LC3, a marker for autophagy. In contrast, IF significantly increased Lc3 expression in the ADR+ Fasting group. Accordingly, Chaudhary et al. (35) showed that IF increased autophagy and Lc3 expression in the liver tissues of C57 BL/6 J mice, but not in skeletal muscles. Numerous studies (36, 37) has produced contradictory findings about the complex role of autophagy in ADR-induced toxicity, which raises the possibility that autophagy may have dual roles in DOX-induced toxicity. It has been shown that protecting against ADR-induced toxicity can be achieved by increasing autophagy, for example, by antioxidant agents or IF, as in our case, before ADR is administered (38). Therefore, we began the IF protocol two weeks prior to the administration of ADR. Thus, by upregulating autophagy, IF in the current study showed a hepatoprotective effect.

Sirt-1 is a member of sirtuin family of proteins with histone deacetylase activity, which is essential for cell growth and metabolism. The Keap1/Nrf2/ARE pathway is specifically regulated by Sirt-1, possibly by reducing Keap1 expression, which enhances Nrf2's transcriptional activity and the ARE's binding capacity (39). Given these concerns, our findings showed that Sirt-1 expression is substantially reduced in the ADR group. However, numerous studies have shown that ADR downregulates AMPK activity, which may be the mechanism behind Sirt-1 downregulation (40). Furthermore, the current investigation showed that IF increased Sirt-1 expression in rats treated with ADR, consistent with other studies reporting that IF significantly elevates Sirt-1 levels and associated metabolic adaptations (41). Additionally, another study discovered that while the IF strategy in conjunction with time-restricted eating-which consists of an 8-hour feeding window and a 16-hour fast could enhance Sirt-1 activity and lower fasting blood glucose levels, it was unable to improve total antioxidants in a diabetes rat-model (42).

Adriamycin-induced Hepatoxicity (ADR) caused significant impairment in liver functions and morphology. This liver injury was associated with oxidative stress, downregulation of antioxidant genes (Nrf2, HO-1), cytoprotective genes (Sirt-1) and autophagy (LC3) with upregulation of caspase-3 and apoptosis. IF may offer hepatoprotective effects against ADR-induced hepatotoxicity which may result from the inhibition of oxidative stress and apoptosis, as well as modulation of Nrf2, LC3, and Sirt-1 expression.

Acknowledgment

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Conflicts of Interest

All authors declared that there was no conflict of interest in this study.

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Authors contribution

A.M.H contribute to the idea, animal model induction, biochemical analyses, revision of the manuscript draft, F.H.E contribute to animal model, biochemical analyses, revision of the manuscript draft, W. E contribute to the biochemical analyses, revision of the manuscript draft, E.A.E contribute to the idea, data analyses, writing of the manuscript draft, O.A.A. contribute to the biochemical analyses and data analyses and manuscript draft writing, A.B contribute to the idea, data analyses, writing of the manuscript draft, G. O. contribute to the idea, data analyses, writing of the manuscript draft, S.E.M. E contribute to the animal model induction, biochemical analyses, revision of the manuscript draft.

References

1. Coldwell KE, Cutts SM, Ognibene TJ, Henderson PT, Phillips DR. Detection of Adriamycin-DNA adducts by accelerator mass spectrometry at clinically relevant Adriamycin concentrations. Nucleic Acids Res. 2008;36(16): e100.

2. Jabłońska-Trypuć A, Świderski G, Krętowski R, Lewandowski W. Newly Synthesized Doxorubicin Complexes with Selected Metals-Synthesis, Structure and Anti-Breast Cancer Activity. Molecules. 2017;22(7):1106.

3. Ahmed OM, Elkomy MH, Fahim HI, Ashour MB, Naguib IA, Alghamdi BS, et al. Rutin and Quercetin Counter Doxorubicin-Induced Liver Toxicity in Wistar Rats *via* Their Modulatory Effects on Inflammation, Oxidative Stress, Apoptosis, and Nrf2. Oxid Med Cell Longev. 2022; 2022:2710607.

4. Cao B, Li M, Zha W, Zhao Q, Gu R, Liu L, et al. Metabolomic approach to evaluating adriamycin pharmacodynamics and resistance in breast cancer cells. Metabolomics. 2013;9(5):960-973.

5. Yapislar H, Taskin E, Ozdas S, Akin D, Sonmez E. Counteraction of Apoptotic and Inflammatory Effects of Adriamycin in the Liver Cell Culture by Clinopitolite. Biol Trace Elem Res. 2016;170(2):373-81.

6. Ahmed OM, Abdul-Hamid MM, El-Bakry AM, Mohammed HM, Rahman FESA. Effects of green tea infusion and epicatechin on doxorubicin-induced renocardiotoxicity in male albino rats. Int J Pharm Sci Res. 2019,10(5),1000-1014.

7. Kim C, Pinto AM, Bordoli C, Buckner LP, Kaplan PC, Del Arenal IM, et al. Energy Restriction Enhances Adult Hippocampal Neurogenesis-Associated Memory after Four Weeks in an Adult Human Population with Central Obesity; a Randomized Controlled Trial. Nutrients. 2020;12(3):638.

8. de Cabo R, Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. N Engl J Med. 2019 Dec 26;381(26):2541-2551. Erratum in: N Engl J Med. 2020;382(3):298.

9. Speakman JR, Mitchell SE. Caloric restriction. Mol Aspects Med. 2011;32(3):159-221.

10. Vemuganti R, Arumugam TV. Much ado about eating: Intermittent fasting and post-stroke neuroprotection. J Cereb Blood Flow Metab. 2021;41(7):1791-1793.

11. Zhou YQ, Mei W, Tian XB, Tian YK, Liu DQ, Ye DW. The therapeutic potential of Nrf2 inducers in chronic pain: Evidence from preclinical studies. Pharmacol Ther. 2021; 225:107846.

12. Fang Y, Chen B, Gong AY, Malhotra DK, Gupta R, Dworkin LD, Gong R. The ketone body β-hydroxybutyrate mitigates the senescence response of glomerular podocytes to diabetic insults. Kidney Int. 2021;100(5):1037-1053.

13. Mattson MP, Longo VD, Harvie M. Impact of intermittent fasting on health and disease processes. Ageing Res Rev. 2017; 39:46-58.

14. Ren Z, He H, Zuo Z, Xu Z, Wei Z, Deng J. The role of different SIRT1-mediated signaling pathways in toxic injury. Cell Mol Biol Lett. 2019; 24:36.

15. Rifaai RA, El-Tahawy NFG, Abozaid SMM, Abdelwahab A. Intermittent Fasting Ameliorates Age-Induced Morphological Changes in Aged Albino Rat Kidney via

[Downloaded from old.rbmb.net on 2026-06-15]

Autophagy Activation and Reduction of Apoptosis and Inflammation. Microsc Microanal. 2025;31(1): ozae102.
16. Abas E, Sabry MM. Intermittent fasting attenuates apoptosis, modulates autophagy and preserves telocytes in doxorubicin induced cardiotoxicity in albino rats: A Histological Study. Egypt J Histol. 2020,43(3): 663-683.
17. Liu H, Javaheri A, Godar RJ, Murphy J, Ma X, Rohatgi N, et al. Intermittent fasting preserves beta-cell mass in obesity-induced diabetes via the autophagy-lysosome pathway. Autophagy. 2017;13(11):1952-1968.
18. Hussein AM, Eldosoky M, Handhle A, Elserougy H, Sarhan M, Sobh MA, et al. Effects of long-acting erythropoietin analog darbepoetin-α on adriamycin-induced chronic nephropathy. Int Urol Nephrol. 2016;48(2):287-97.
19. Ibrahim HAM, Hussein AM, Gabr M, El-Saeed RA, Ammar OA, Mosa AAH, Abdel-Aziz AF. Effect of Melatonin on Alpha Synuclein and Autophagy in Dopaminergic Neuronal Differentiation of Adipose Mesenchymal Stem Cells. Rep Biochem Mol Biol. 2023;12(1):13-26.
20. Kaya S, Yalcın T, Tektemur A, Kuloğlu T. N-Acetylcysteine may exert hepatoprotective effect by regulating Meteorin-Like levels in Adriamycin-induced liver injury. Cell Stress Chaperones. 2023;28(6):849-859.
21. Prasanna PL, Renu K, Valsala Gopalakrishnan A. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. Life Sci. 2020; 250:117599.
22. Renu K, Pureti LP, Vellingiri B, Gopalkrishnan AV. Toxic effects and molecular mechanism of doxorubicin on different organs–an update. Toxin Rev.2022, 41(2): 650-674.
23. Kaymak E, Öztürk E, Akİn AT, Karabulut D, Yakan B. Thymoquinone alleviates doxorubicin induced acute kidney injury by decreasing endoplasmic reticulum stress, inflammation and apoptosis. Biotech Histochem. 2022;97(8):622-634.
24. Zobeydi AM, Mousavi Namavar SN, Sadeghi Shahdani M, Choobineh S, Kordi MR, Rakhshan K. Mitigating doxorubicin-induced hepatotoxicity in male rats: The role of aerobic interval training and curcumin supplementation in reducing oxidative stress, endoplasmic reticulum stress and apoptosis. Sci Rep. 2025;15(1):6604.
25. Longo VD, Mattson MP. Fasting: molecular mechanisms and clinical applications. Cell Metab. 2014;19(2):181-92.
26. Al-Kazimi N, Jarrar Y, Abdul-Wahab G, Alsayed AR, Madani A, Abulebdah D, et al. Effects of intermittent fasting on the histology and mRNA expression of major drug-metabolizing cyp450s in the liver of diabetic mice. Libyan J Med. 2023;18(1):2270188.
27. Costa Godinho LRL, Cella PS, Guimarães TAS, Palma GHD, Nunes JHC, Deminice R. Creatine Supplementation Potentiates Exercise Protective Effects against Doxorubicin-Induced Hepatotoxicity in Mice. Antioxidants (Basel). 2023;12(4):823.
28. Hardiany NS, Karman AP, Calista ASP, Anindyanari BG, Rahardjo DE, Novira PR, et al. The Effect of Fasting on Oxidative Stress in the Vital Organs of New Zealand White Rabbit. Rep Biochem Mol Biol. 2022;11(2):190-199.
29. Elsaid FH, Hussein AM, Eid EA, Ammar OA, Khalil AA. Effect of intermittent fasting on adriamycin-induced nephropathy: Possible underlying mechanisms. Tissue Cell. 2024; 88:102360.
30. Obydah W, Abouelnaga AF, Abass M, Saad S, Yehia A, Ammar OA, at al. Possible Role of Oxidative Stress and Nrf2/HO-1 Pathway in Pentylenetetrazole-induced Epilepsy in Aged Rats. Rep Biochem Mol Biol. 2023;12(1):147-158.
31. Almeldin A, Younis R, Ibrahim RR, Motawea Sh, Mwafy M, Khattab HA. Egyptian Propolis Extract Attenuates Hepatotoxicity Induced by Doxorubicin via Increasing Antioxidant Defense and Decreasing Inflammatory and Apoptotic Markers: Targeting Nrf2 and Bcl-2. Bull Egypt Soc for Physiol Sci.2024, 44(2),69-82.
32. Khodir SA, Nagy A, Abd El-aziz N, Mohamed A, Hussein S, Elgheriany W, et al. Hesperidin mitigates Doxorubicin-induced hepatic toxicity in rats, targeting JAK-STAT

[Downloaded from old.rbmb.net on 2026-06-15]

signaling pathway. Mansoura J Forensic Med Clin Toxicol. 2025, 33(1): 33-43.
33. Shabkhizan R, Haiaty S, Moslehian MS, Bazmani A, Sadeghsoltani F, Saghaei Bagheri H, et al. The Beneficial and Adverse Effects of Autophagic Response to Caloric Restriction and Fasting. Adv Nutr. 2023;14(5):1211-1225.
34. Ma YN, Jiang X, Tang W, Song P. Influence of intermittent fasting on autophagy in the liver. Biosci Trends. 2023;17(5):335-355.
35. Chaudhary R, Liu B, Bensalem J, Sargeant TJ, Page AJ, Wittert GA, et al. Intermittent fasting activates markers of autophagy in mouse liver, but not muscle from mouse or humans. Nutrition. 2022; 101:111662.
36. Gu J, Fan YQ, Zhang HL, Pan JA, Yu JY, Zhang JF, Wang CQ. Resveratrol suppresses doxorubicin-induced cardiotoxicity by disrupting E2F1 mediated autophagy inhibition and apoptosis promotion. Biochem Pharmacol. 2018; 150:202-213.
37. Johnson R, Shabalala S, Louw J, Kappo AP, Muller CJF. Aspalathin Reverts Doxorubicin-Induced Cardiotoxicity through Increased Autophagy and Decreased Expression of p53/mTOR/p62 Signaling. Molecules. 2017;22(10):1589.
38. Koleini N, Kardami E. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. Oncotarget. 2017;8(28):46663-46680.
39. Iside C, Scafuro M, Nebbioso A, Altucci L. SIRT1 Activation by Natural Phytochemicals: An Overview. Front Pharmacol. 2020; 11:1225.
40. El-Dessouki AM, Yousef EH, Raslan NA, Alwakeel AI, Ibrahim S, Alzokaky AA. Febuxostat protects from Doxorubicin induced hepatotoxicity in rats via regulation of NF-κB p65/NLRP3 inflammasome and SIRT-1/AMPK pathways. Naunyn Schmiedebergs Arch Pharmacol. 2025;398(8):10125-10137.
41. Gentles TL, Levitsky Y, Fisher K, Hammer S, Busik J, Crockett E, Proshlykov D. SIRT1 Activation and mitochondrial dynamics in Retinal Endothelial Cells. Invest Ophthalmol Vis Sci. 2020, 61(7): 729-729.
42. Safitri M, Harliansyah H, Wuryanti S. Effect of intermittent fasting on fasting blood glucose, sirtuin 1, and total antioxidant capacity in rat models of diabetes mellitus. Jurnal Kedokteran dan Kesehatan Indonesia. 2024, 15 (1): 27-36.

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