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Research Article
Potential Chemotherapeutic Effect of Coenzyme Q10 Against Liver Injury in a Leukemia Rat Model by 7,12-Dimethylbenz[a]anthracene

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Abstract

Background and Objective: Coenzyme Q10 (CoQ10) is an essential cofactor in the mitochondrial electron transport chain that benefits liver disorders. The polycyclic aromatic hydrocarbon (PAH) 7,12-dimethylbenz[a]-anthracene (DMBA) originates and promotes carcinogenesis. This study investigated the potential anti-leukemic effects of CoQ10 in a DMBA-induced leukemic rat model. **Materials and Methods:** Forty adult male albino rats were split equally into four groups: Control group (Group I), coenzyme Q10 group (Group II), DMBA group (Group III) and coenzyme Q10 plus DMBA group (Group IV). The first DMBA dose of 40 mg/kg body weight produced leukaemia in all rats except GI and GII rats. Three further injections were given at a rate of 30 mg/kg body weight every two weeks for a total of 6 weeks. Body weight and blood samples were evaluated once the experiment was complete. Biochemical, histological, immunohistochemical and morphometric analysis were performed on liver tissue. **Results:** The CoQ10 treatment significantly reduced liver enzyme levels relative to leukemia-model animals. Malondialdehyde (MDA) levels were much lower in the CoQ10 group compared to the DMBA group, whereas Glutathione (GSH) and superoxide dismutase (SOD) levels were significantly increased. Histopathological examination of rat DMBA livers revealed a loss of normal hepatic architecture, which was restored after administration of CoQ10. **Conclusion:** Treatment with CoQ10 attenuated the alleviation of DMBA-induced leukemia in rats, claiming its potential as an effective therapy for leukaemia and liver impairment caused by leukaemia.

Key words: CoQ10, 7,12-dimethylbenz[a]-anthracene, liver, leukemia, rats

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Leukemia is one of the worst forms of cancer affecting the bone marrow or blood of people of any age. Unlike most malignancies, which start in the body's primary organs and then move to the bone marrow, leukaemia tends to behave in a unique way[1,2]. Many different forms of leukaemia were recognized, but the most common four were Acute Myeloid Leukaemia (AML), Chronic Myeloid Leukaemia (CML), Acute Lymphoblastic Leukaemia (ALL) and Chronic Lymphocytic Leukaemia (CLL). Acute lymphoblastic leukaemia is the most aggressive kind of leukaemia and it is also the most fatal[2,3]. Leukemia was classified as the 13th most common disease in the world by a GLOBOCAN study in 2018 and leukemia mortality climbed by 17% in the same year[4-6].

Creating a disease model and verifying newly identified treatment targets both require an *in vivo* model of blood cancer, making its development a prerequisite for clinical trials. As a result, numerous attempts employing various leukemogenic substances to cause leukemia have been made.

Pollutant Polycyclic Aromatic Hydrocarbons (PAHs) can be found in cooked foods and are widely spread throughout the environment. Daily human exposure to PAHs is inevitable[7,8]. The PAHs are poisonous, mutagenic and carcinogenic, all of which have negative impacts on biological systems. The carcinogenic synthetic PAH 7,12-dimethylbenz[a]-anthracene (DMBA) is well-known for its role in initiating and promoting carcinogenesis, a process that underpins the emergence of numerous tumor types, such as those of the mammary gland, skin, liver, lung, haematological system and pancreas[9-13].

The liver is the primary metabolic organ that controls energy metabolism and it is particularly vulnerable to xenobiotic injury[14,15]. To become fully carcinogenic, DMBA must first undergo metabolic activation in the liver and extra-hepatic organs[16,17]. Liver function biomarkers such as Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Gamma-Glutamyl Transferase (GGT) are routinely tested, although it is unknown whether they can accurately depict cancer progression and serve as diagnostic or prognostic tools. This normal hepatic biomarker, however, has been demonstrated in multiple studies to have predictive value in diseases other than those originating in the liver, including type II diabetes and cardiovascular disease[18,19]. So, it should be expected that this biomarker also has biomedical significance in cancer. Thus, clarifying the mechanisms of liver injury under DMBA-induced leukemia in a rat model and finding treatment for such conditions is critical.

The mitochondria produce the lipid-soluble quinone Coenzyme Q10 (CoQ10), also known as ubiquinone, which is essential for the mitochondrial electron transport chain[20]. The CoQ10's key roles are in antioxidant defence, membrane stabilisation and ATP generation during aerobic respiration. Ubiquinol, is the unique lipid soluble antioxidant that can be synthesised de novo by animal cells; it works as a lipophilic antioxidant, limiting the start and/or propagation of free radicals and lipid peroxidation in biological membranes[21,22].

The CoQ10 has several medicinal applications due to its antioxidant properties and its function in ATP production. The CoQ10 supplementation has been linked to complete remission from cancer in some high-risk instance[23]. The CoQ10 supplementation alongside chemotherapy and radiation has been linked to total elimination of metastases in some cases[24]. The CoQ10 also has multiple positive impacts on liver disorders. It reduces inflammation and corrects dysregulated lipogenesis in non-alcoholic fatty liver disease[25,26].

The CoQ10's potential chemo-preventive action against DMBA-induced liver injury has not been studied to this point. Hence the current study was designed to investigate the potential anti-leukemic effects of CoQ10 in a DMBA-induced leukemic rat model. In addition, the biochemical, histological and immunohistochemical analysis of the liver was also assessed to determine that CoQ10 would modify liver injury under DMBA-induced Leukemia in a rat model.

MATERIALS AND METHODS

Study area: The study was carried out in 2022 in King Fahad Medical Research Center at King AbdulAziz University, Saudi Arabia.

Ethical statement: The experimental protocols had been approved by the Institutional Animal Care and Use Committee, Kind Abdulaziz University, Jeddah, Saudi Arabia. The number of animals utilized was cut down on and pain was kept to a minimum. All procedures were conducted rapidly and carefully to reduce the likelihood of stress-related changes. All animal procedures were carried out by the ethical standards set forth by the National Institutional Animal Care and Use Committee and the Committee for the Control and Supervision of Experiments on Animals.

Drugs: The 7,12-dimethylbenz[a]-anthracene (DMBA, Cat. No. 57-97-6)) and Coenzyme Q10 (CoQ10, Cat. No. 57-97-6) were purchased from Sigma-Aldrich Chemical Co., Saint Louis, Missouri, USA, Cat. No. 303-98-0).

Experimental animals: Forty adult male albino rats weighing between 180 and 250 g were used in the study. They were obtained from King Fahd Animal House, Jeddah, Saudi Arabia. The rats were kept in standard animal cages and provided with running water. After a one-week acclimatization period, four groups of rats were divided randomly.

Leukemia induction using DMBA: Rats were given freshly dissolved DMBA that had been pre-warmed to 37°C by intravenous injection twice a week to artificially induce leukaemia. Initial dosing was 40 mg/kg body weight, administered as an emulsion in DMSO (enhanced with olive oil) at a concentration of 20 mg/mL. Three further injections were given at 2 weeks intervals for a total of 6 weeks, at a dose of 30 mg/kg body weight[27]. The DMSO group also gave their rats the same method of administration as the vehicle group. Weight loss, the number of leucocytes in a blood sample taken from the tail vein and the presence of malignant blasts and atypical lymphocytes were all used to diagnose leukaemia.

Study design and treatment: The forty experimental rats were split into four groups of ten and housed in cages at random. Group I (Control group): Half of the rats in this group were healthy (subgroup Ia) and the other half were normal (subgroup Ib) after receiving intravenous injections of DMSO twice weekly for 6 weeks (Ib). For 10 weeks, rats in Ia and Ib were maintained on a standard diet and water. In Group II (CoQ10 group), normal rats were given DMSO through intravenous injection biweekly for 6 weeks, followed by 4 weeks of daily gastro intubation doses of 200 mg/kg CoQ10[28]. Group III (DMBA group) included the DMBA-induced leukemic group[27]. Group IV (CoQ10+DMBA group) included DMBA-induced leukemic rats for 6 weeks then received CoQ10 as treatment for 4 weeks.

At the outset of the experiment (at time zero), the weight of each rat in all groups was recorded and subsequent weekly weights were also recorded until the end of the study. The change in body weight was calculated after the last week using the equation:

$$\text{Body weight gain (\%)} = \frac{\text{Final weight-Initial weight}}{\text{Initial weight}} \times 100$$

All animals were put to death at the conclusion of the trial period under minimal doses of ketamine and xylazine. A cardiac puncture was used to draw blood and the liver was quickly removed for examination. The left lobe of the liver was dissected and cut in half so that one half could undergo standard histological and immunohistochemical preparation. While the other half was getting ready to be homogenised liver.

Blood collection for White Blood Cell (WBC) count: Blood was drawn and stored in EDTA tubes. Immediate preparation, air drying and staining per manufacturer's directions were used on the blood smears. After diluting and staining with Gentian violet, the total Weight Blood Cells (WBCs) count was taken with a haemocytometer (Countess™ 3 Automated Cell Counter Package, ThermoFisher, USA, Cat. #: A49865) per normal procedure. Leishman's staining and examination of blood films under a 100× oil immersion lens constitute the standard procedure for WBC differential count.

Liver function assessment: The blood samples were centrifuged for 15 min at 4°C and 10,000 rpm. Separated plasma was placed in 1.5 mL Eppendorf tubes and frozen at 20°C for later use. The activity of liver function was evaluated with serum levels of Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) to determine liver damage. Spectrum Diagnostics® commercial colorimetric kits (Cat. #: 264001 and 260001, respectively, Al-Obour City, Cairo, Egypt) were used to evaluate all biochemical assays, with results interpreted by the manufacturer's instructions.

Preparation of liver tissue homogenate: Liver samples (10% w/v) were homogenized in ice-cold 0.1M Tris-HCl buffer (pH 7.4). Then centrifuged (3000 rpm for 10 min at 4°C) and the supernatant was used to determine markers of oxidative stress. The hepatic malondialdehyde (MDA) and reduced Glutathione (GSH) and superoxide dismutase (SOD) contents were determined via biochemical kits purchased from Sigma-Aldrich Chemical Co., Saint Louis, Missouri, USA (Cat. #: MAK085 and 70-18-8, 9054-89-1, respectively).

Histopathological examinations: Formalin at a 10% concentration was used to preserve all liver samples. The specimens were subsequently dehydrated using 70, 95 and 100% alcohol, cleaned using xylene and finally imbedded in paraffin wax. Leica rotator microtomes (Germany) were used to slice paraffin blocks to a thickness of 5 µm. These sections were stained for light microscopic (Olympus BX61-USA) examination. Paraffin sections were stained with Haematoxylin and Eosin (H&E) to evaluate liver architectural changes and Masson Trichrome stain (a special stain for collagen fibers) to determine the degree of fibrosis[29].

Immunohistochemical staining of BCL-2 antiapoptotic gene (BCL-2): After being counter in xylene and rehydrated in categorized alcohol, liver tissue slices were heated in an oven at 60°C for 30 min. The antigen recovery technique utilized a microwave-boiled sodium citrate buffer of 10 mM. The staining method was demonstrated by the manual provided by the supplier. Briefly, slices were treated with a 0.03% sodium azide hydrogen peroxide solution for 5 min to hunk endogenous peroxidase. Slices were washed, then treated with biotinylated primary antibodies against BCL-2 (BCL-2, Cat. #: ab194583, Abcam®, Cambridge, UK) for 15 min, followed by incubation with rabbit anti-rat BCL-2 antibody (1:100) for 30 min. After a brief rinsing in distilled water for 3 min, tissue slices were stored in a buffer bath in a humid area. Slices were treated with streptavidin HRP, incubated for 15 min and then rinsed twice in distilled water for 3 min. After soaking biopsy sections in diaminobenzidine (DAB) substrate chromogen for 5 min, washed for 5 sec in to counterstain. After being submerged in weak ammonia (0.037 M/L) ten times, rinsed with distilled water for 2-5 min and mounted on a rising medium, the slices were evaluated. Positive antigens show up as brown stains on a blue haematoxylin background when viewed via a compound microscope.

Morphometric study: At least five animals per experimental group had sections investigated for quantitative histological and immunohistochemical analysis. Each group's images were taken using a Leica Microscope DML B2/11888111 and a Leica camera DFC450, randomly capturing five non-overlapping fields in each section. Morphometric evaluations were performed on a sampling of liver sections using image analysis programs (ImageJ analyzer version 1.43o8, National Institutes of Health, USA). It was determined by measuring these factors. Mallory trichrome staining for collagen fiber area % (×20) and BCL-2 positive immune staining intensity (×40).

Statistical analysis: The computer application SPSS was used for all statistical analyses (Statistical Packages for Social Sciences, version 26.0). One-way Analysis of Variance (ANOVA) was used to assess biochemical and morphometric data and then the least significant difference (LSD) multiple comparisons test was performed when the p-value showed overall significance. Body weight data analysis was compared among various groups by two-way ANOVA with *post hoc* Tukey's multiple comparisons test when significant differences were found. The data is presented as the Mean±Standard Deviation (SD). Statistical significance was set at the $p<0.05$ level. The charts were generated in GraphPad Prism version 10 (Graph Pad® Inc., USA).

RESULTS

Effect of CoQ10 supplementation on body weight of DMBA-induced leukemic rats: According to the findings of the current study, the DMBA group had a significant weight loss over the course of the experiment's 10 weeks. Group III demonstrated the greatest drop in DMBA (leukemia rat model) relative to both the control and Group IV (Fig. 1a).

There was a statistically significant difference in mean body weight between GI and GII rats, DMBA-treated rats (GIII) and DMBA-treated rats given CoQ10 (GIV) during the course of the study's main weeks, as shown in Fig. 1b, two-way ANOVA followed by Tukey's multiple comparisons tests (week 0: Start of the experiment, week 6: Beginning of the treatment and week 10: End of the experiment). Rats of the control and CoQ10 groups showed an increase in body weight from week 0 (200.60±12.39 and 203.40±11.10 g, respectively) to the end of the experimental period (234.80±10.39 and 219.20±13.00 g, respectively). A significant decrease in body weight gain ($p<0.0001$, $p = 0.0360$, respectively) was observed in the DMBA group, starting at the sixth week (196.20±12.83 g) and the DMBA+CoQ10 group (196.20±12.83 g) compared to control group and CoQ10 (226.40±10.55 and 211.70±13.54 g, respectively). A highly significant ($p<0.0001$) decrease in the body weight of the DMBA rats (GIII) (183.80±12.21 g) was observed when compared with the corresponding control group (GI, 234.80±10.39 g) and CoQ10 (GII, 219.2±13.00 g) at the end of the experiment (week 10). However, CoQ10 rind effectively ameliorated DMBA (leukemia rat model)-induced weight loss. In CoQ10+DMBA group (GIV, 205.90±11.99 g), the body weight was significantly ($p = 0.0008$) higher than DMBA group (GIII, 183.80±12.21 g) at week 10. The rats' body weight gain decreased ($p<0.0001$) significantly in the DMBA group with an average value of (8.33±1.86%) compared to the control group (17.27±4.99%) at the end of the experiment (Fig. 1b).

Leukemia diagnosis: Total White Blood Cell (WBC) count, lymphocyte percentage, neutrophil percentage and blast percentage were compared between the DMBA rat group (GIII) and the control rat group (GI) to determine the efficacy of DMBA in inducing leukaemia in rats (Fig. 2a-d). The typical value of lymphocytes in a rat is 60-75%, while the normal range for leukocyte count is 2000-10000 cells/μL[30].

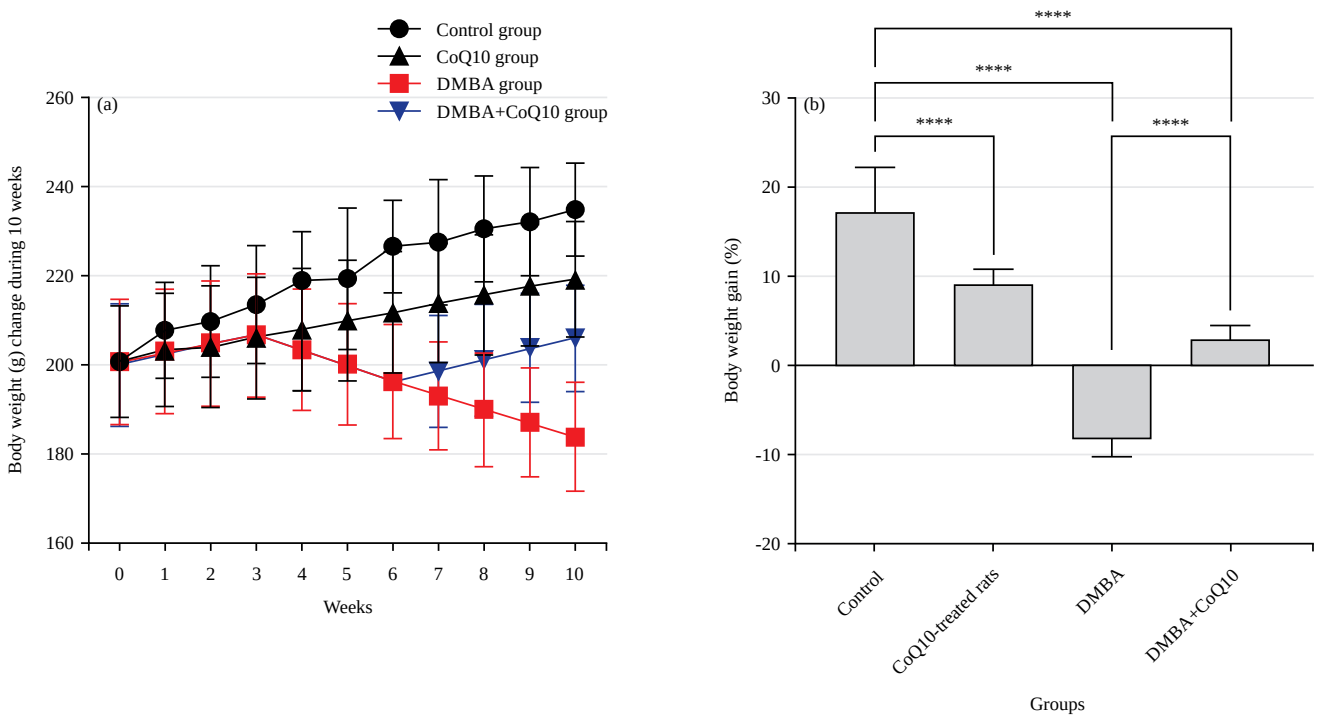


Fig. 1(a-b): (a) Variation of body weight (g) in rats with DMBA-induced leukemia and treated with CoQ10 for 10 weeks and (b) Percentage of body weight gain for the control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+CoQ10-treated rats (G IV) groups (n = 10 rat/group)
Significance at ****p<0.0001

The results of the current study showed that the total WBC count in the GI (control) and GII (CoQ10) groups (2700±537.50 and 2650±668.7 cells/μL, respectively) remained within normal ranges, the total WBC count was statistically lower (p<0.0001 and p = 0.03) in contrast with other rats in DMBA (GIII) and DMBA+CoQ10 (GIV) groups (47600±5275 and 6100±1578 cells/μL, respectively).

The GI group was controlled with lymphocyte count (%) 61.60±10.06 at the end of the experiment. The GIII group of DMBA leukemic rats model group had lymphocyte counts (%) of 30.80±4.39. However, CoQ10 rind effectively ameliorated DMBA (leukemia rat model)-induced increase of lymphocyte count (%). In the GIV group CoQ10+DMBA group, 49.70%±7.87), the lymphocyte count (%) was significantly (p<0.0001) higher than the DMBA group (GIII) at week 10. There was a non-significant (p = 0.99) difference between GII (CoQ10; 62.10±10.37) and GI (control group).

At the end of the experiment, GI and GII groups exhibited neutrophil count (%) of 25.30±4.42 and 26.30±3.49, respectively. The group of leukemic rats' model DMBA (GIII) had a neutrophil count (%) of 16.70±2.35. However, CoQ10 rind effectively ameliorated DMBA (leukemia rat model)-induced increase of neutrophils count (%). In the CoQ10+DMBA group (GIV, 21.00±3.16%), the neutrophils count (%) was significantly (p = 0.0327) higher than the DMBA group (GIII) at week 10. There was a non-significant (p = 0.9469) difference between the CoQ10 group (GII) and the control group (GI).

At the end of the experiment, GI and GII groups exhibited Blasts count (%) 1.30±0.48 and 1.50±0.52, respectively. The group of leukemic rats' model DMBA (GIII) had Blasts count (%) of 29.80±3.853. However, CoQ10 rind effectively ameliorated DMBA (leukemia rat model)-induced decrease of Blasts count (%). In CoQ10+DMBA group (GIV, 4.20±1.68%), the Blast count (%) was significantly (p<0.0001) lower than the DMBA group (GIII) at week 10. There was a non-significant (p = 0.99) difference between the CoQ10 group (GII) and the control group (GI).

Assessment of the effect of CoQ10 on DMBA induced leukemia rat model on serum liver enzymes: As shown in Fig. 3a, the results of the current study showed that the DMBA group exhibited a significant (p<0.0001) increase in ALT level (76.90±9.06 IU/L) as compared to the

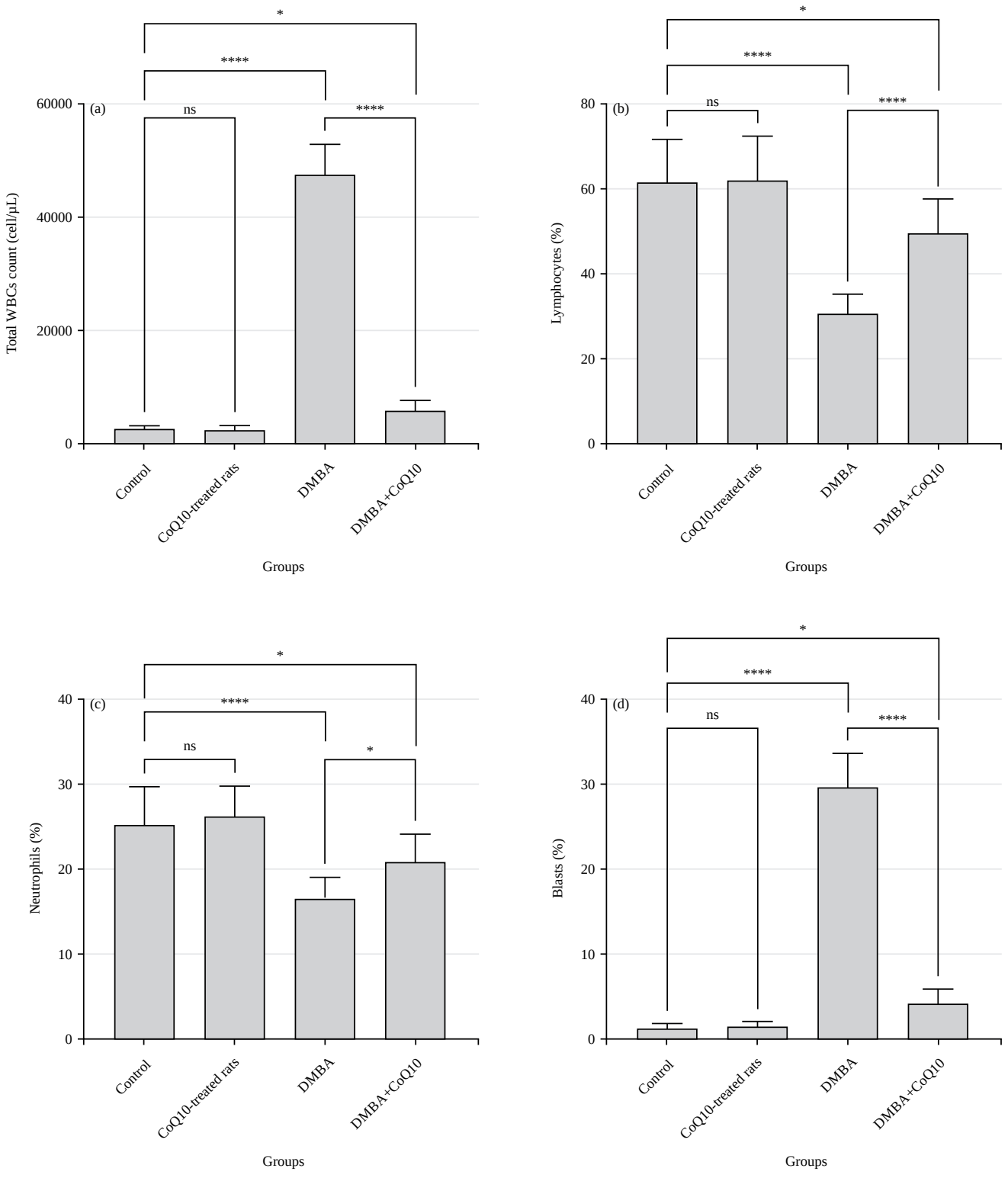


Fig. 2(a-d): Mean±Standard Deviation (SD) of (a) Total WBCs (cells/µL), (b) Lymphocytes (%), (c) Neutrophils (%) and (d) Blasts (%) for the control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+Co Q10-treated rats (GIV) groups (n = 10 rat/group)

ns: Non-significant, Significant at *p<0.05 and ****p<0.0001

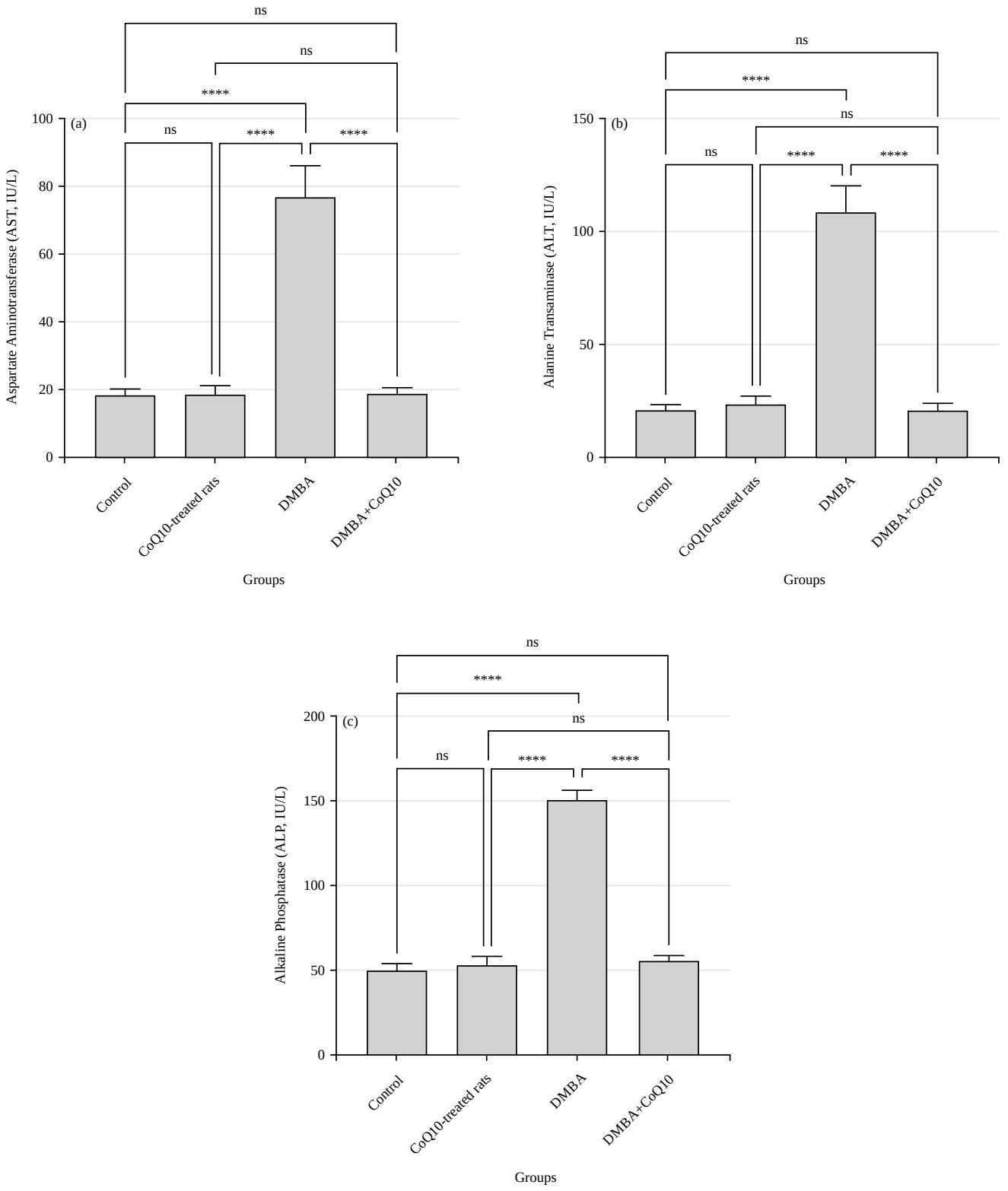


Fig. 3(a-c): Mean±Standard Deviation (SD) of (a) Aspartate Aminotransferase (AST, IU/L), (b) Alanine Transaminase (ALT, IU/L) and (c) Alkaline Phosphatase (ALP, IU/L) levels for the control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+Co Q10-treated rats (GIV) groups (n = 10 rat/group)

ns: Non-significant and Significant at ****p<0.0001

control group (18.48±1.68 IU/L). Interestingly, after CoQ10 treatment, the rats showed a significant ($p<0.0001$) decrease in ALT levels (19.30±1.31 IU/L) compared to the DMBA group. In addition, there was a non-significant ($p = 0.99$ and $p>0.99$, respectively) increase in ALT levels in rats in DMBA+CoQ10 in contrast with the control and CoQ10 (18.58±2.61 IU/L) groups.

As shown in Fig. 3b, the results showed that the DMBA group exhibited a significant ($p<0.0001$) increase in AST level (108.8±11.54 IU/L) in contrast with the control group (21.10±2.28 IU/L). Interestingly, after CoQ10 treatment, the rats achieved a significant ($p<0.0001$) decrease in AST levels (21.56±2.41 IU/L) compared to the DMBA group. In addition, there was a non-significant ($p>0.99$ and $p = 0.97$, respectively) increase in AST levels in rats in DMBA+CoQ10 in contrast with the control and CoQ10 (23.72±3.53 IU/L) groups.

As shown in Fig. 3c, the results showed that the DMBA group exhibited a significant ($p<0.0001$) increase in ALP level (150.70±5.61 IU/L) in contrast with the control group (50.00±3.71 IU/L). Interestingly, after CoQ10 treatment, the rats achieved significantly ($p<0.0001$) decreased ALP levels (55.40±3.06 IU/L) in contrast with the DMBA group. In addition, there was a non-significant ($p = 0.06$ and $p = 0.83$, respectively) increase in ALP levels in rats in DMBA+CoQ10 compared to the control and CoQ10 (53.10±4.97 IU/L) groups.

Assessment of the effect of CoQ10 on DMBA induced leukemia rat model on oxidative stress parameters in liver homogenate: As shown in Fig. 4a, the results showed that the DMBA group exhibited a significant ($p<0.0001$) increase in MDA level (4.799±1.68 nmol/g) in contrast with the control group (1.10±0.74 nmol/g). Interestingly, after CoQ10 treatment, the rats achieved a significant ($p<0.0001$) decrease in MDA levels (2.06±0.56 nmol/g) in contrast with the DMBA group. In addition, there was a non-significant ($p = 0.32$ and $p>0.99$, respectively) increase in MDA levels in rats in DMBA+CoQ10 in contrast with the control and CoQ10 (2.01±1.11 nmol/g) groups.

As shown in Fig. 4b, current results showed that the DMBA group exhibited a significant ($p<0.0001$) decrease in GSH level (2.46±0.89 ng/mg) in contrast with the control group (18.54±2.44 ng/mg). Interestingly, after CoQ10 treatment, the rats achieved a significant ($p<0.0001$) increase in GSH levels (19.46±1.31 ng/mg) in contrast with the DMBA group. In addition, there was a non-significant ($p = 0.78$ and $p = 0.14$, respectively) increase in GSH levels in rats in DMBA+CoQ10 in contrast with the control and CoQ10 (17.73±1.63 ng/mg) groups.

As shown in Fig. 4c, current results showed that the DMBA group exhibited a significant ($p<0.0001$) decrease in SOD level (87.57±8.25 U/g) in contrast with the control group (140.60±9.07 U/g). Interestingly, after CoQ10 treatment, the rats achieved significantly ($p<0.0001$) increased SOD levels (151.20±10.28 U/g) in contrast with the DMBA group. In addition, there was a non-significant ($p>0.99$ and $p = 0.97$, respectively) increase in SOD levels in rats in DMBA+CoQ10 in contrast with the control and CoQ10 (143.10±11.65 U/g) groups.

Histopathological results

Haematoxylin and Eosin (H&E) stain: No histological differences were observed in the two subgroups of the control group (GI) and COQ10 group (GII). Histological sections stained by H&E of GI (Fig. 5a-c) and GII (Fig. 5d-f) showed the normal hepatic structure. There are several typical hepatic lobules, with cords of hepatocyte plates extending out from the lobules' central veins. Blood sinusoids divide the cell cords. The hepatocytes appeared polygonal, with their sides touching either the blood sinusoids or neighboring cells. They contained eosinophilic cytoplasm and central vesicular nuclei, each of which featured one or two conspicuous nucleoli. Binucleation was uncommon among hepatocytes. Endothelial cells and phagocytic Kupffer cells surrounded the blood sinusoids. Even the central veins had endothelial cells lining their interiors. It was also found that portal tracts had hepatic arteries, portal vein and branches of bile ducts.

Liver sections from DMBA-treated rats (GIII) showed vascular and degenerative alterations (Fig. 5g-i). Hepatic tissue structure was markedly disrupted and the hexagonal hepatocyte morphology was lost, as part of a structural degenerative process of varying severity. Areas of highly eosinophilic cells seemed to have fused together, their borders blurred and their nuclei are strongly stained. Some abnormal, degenerating hepatocytes showed up with vacuolated cytoplasm. Meanwhile, cells with shattered nuclei began to show up. Periportal hepatocytes showed necrotic alterations as well. The interstitial spaces between the liver cells were filled with a consistent acidophilic material. Most central veins seemed enlarged and clogged, indicating that vascular alterations had taken place. There was a noticeable clustering of activated Kupffer cells in the Disse space. Some core veins were found to have thickened wall structures and a layer of highly eosinophilic cells with darkly coloured nuclei. Leukocyte aggregations were found in the portal tract and in focal locations between the hepatocytes. There was a dilated, clogged and obstructed portal vein with a broken wall in the portal tract regions. The blood sinusoids were clogged and enlarged.

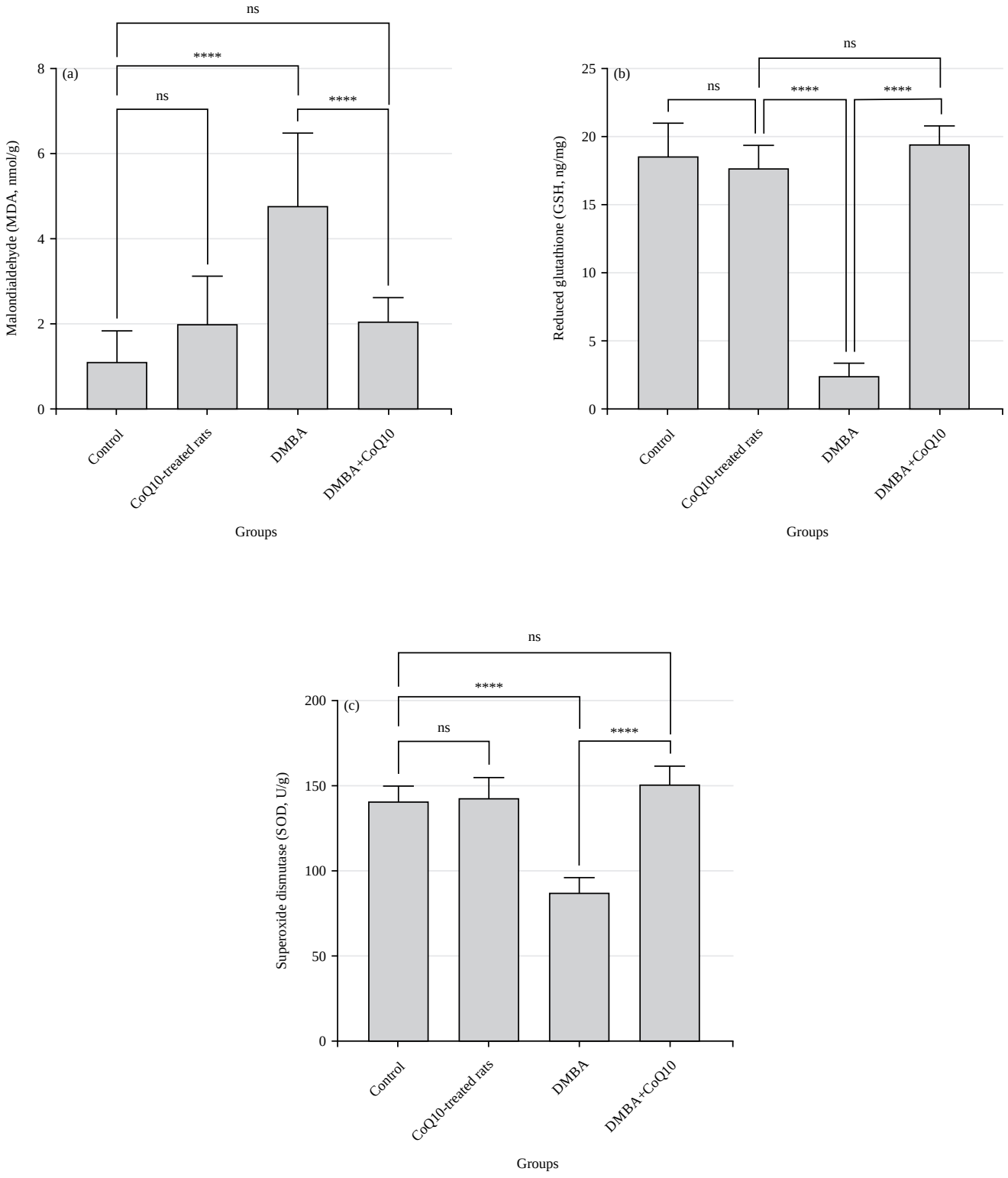


Fig. 4(a-c): Mean±Standard Deviation (SD) of (a) Malondialdehyde (MDA, nmol/g), (b) Reduced glutathione (GSH, ng/mg) and (c) Superoxide dismutase (SOD, U/g) levels for the control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+Co Q10-treated rats (GIV) groups (n = 10 rat/group)

ns: Non-significant and Significant at ****p<0.0001

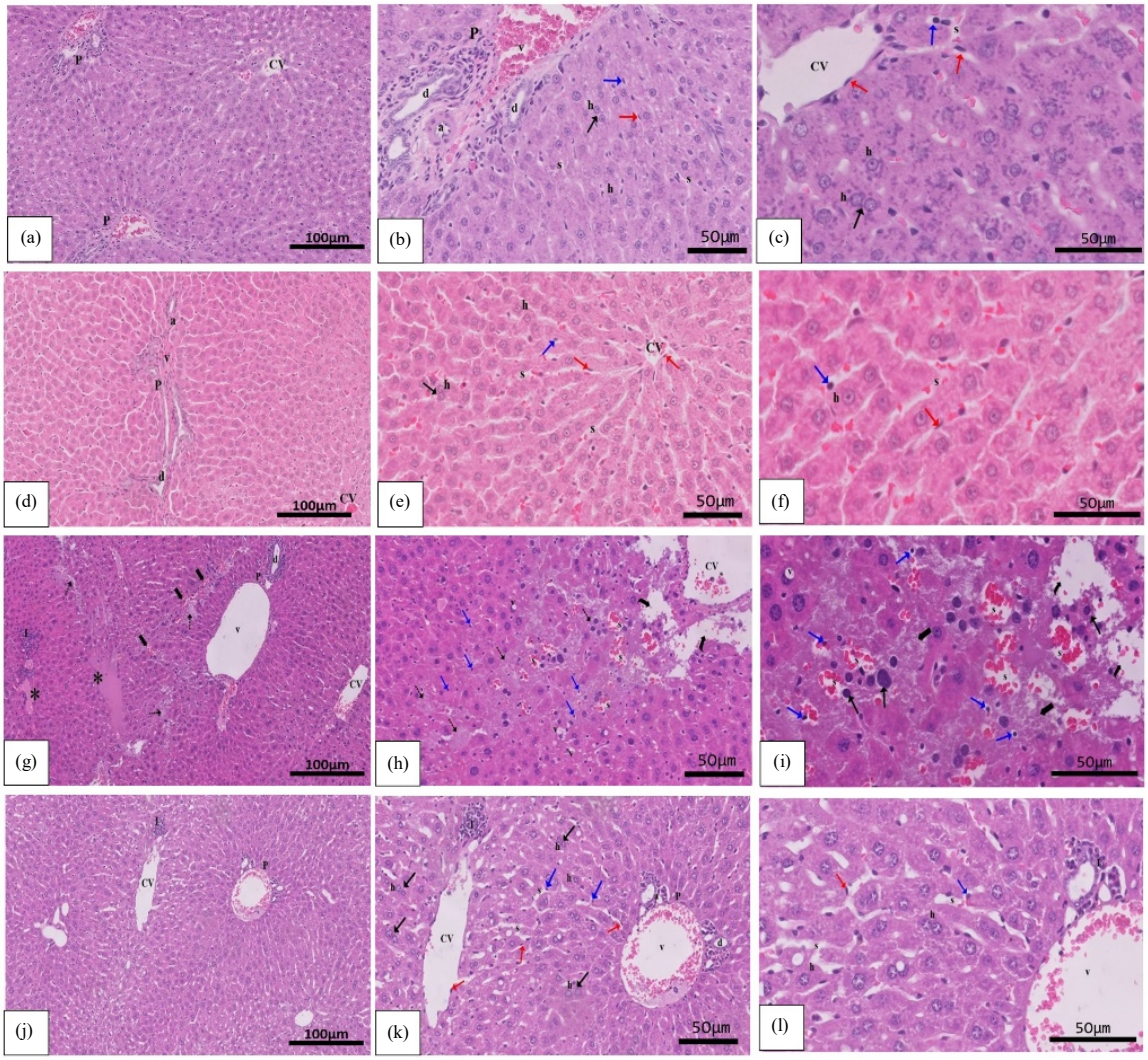
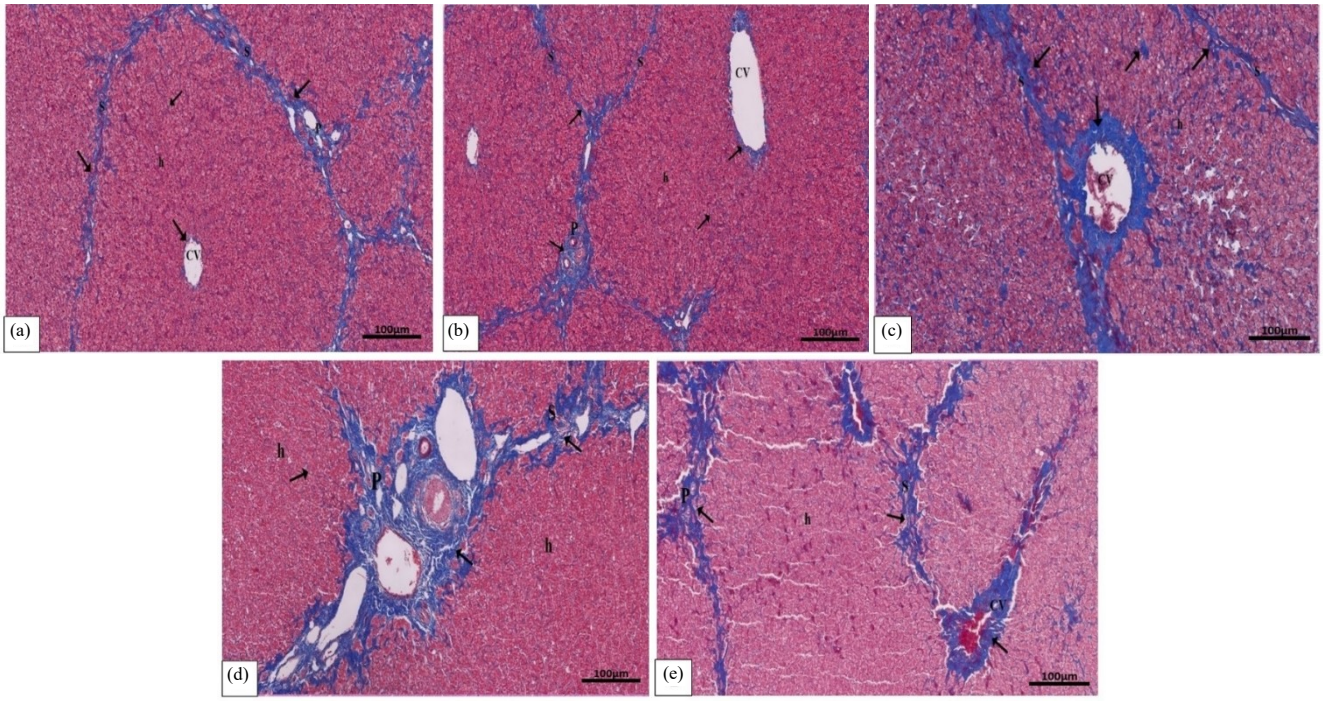


Fig. 5(a-l): Photomicrographs of sections of rat liver from, (a-c) Control group (GI), (d-f) CoQ10-treated rats (GII), (g-i) DMBA group (GIII) and (j-l): DMBA+CoQ10 group (GIV)

(a-c) Show the normal hepatic structure formed of plates of hepatocytes arranged in cords, radiating from the central vein (CV) to the periphery of the lobules. The cell cords were separated by blood sinusoids (s) which are lined by endothelial cells (red arrow) and phagocytic Kupffer cells (blue arrow). The hepatocytes (h) have eosinophilic cytoplasm and central large vesicular nuclei with prominent nucleoli, where each nucleus containing one or two nucleoli. Few hepatocytes were binucleated (↑). Portal tracts (P) containing branches of hepatic artery (a), vein (v) and bile duct (d) are also detected, which were lined by simple cubical epithelial cells. (d-f) Showed no histological difference from control group GI. (g-i) Show structural degenerative changes (curved arrow) of variable severity and sever hemorrhagic area (Thick arrow). Patchy areas of highly eosinophilic cells near to central vein (CV). Some degenerative hepatocytes appear with cytoplasmic vacuolation (v). Also, necrotic areas (dot arrow) are observed with hepatocytes with remnants of cytoplasm and lost their nuclei. Areas of homogenous acidophilic (*) substance are seen in between hepatocytes. Most of central veins (CV) and blood sinusoids (s) appear dilated and congested. Aggregation of activated Kupffer cells (blue arrow) in the space of Disse are seen. Focal areas of leucocytic (l) aggregations infiltrating in-between hepatocytes and bile duct (d) are present. The portal tract (P) areas show dilated congested portal vein (v) with interrupted wall. (j-l) Show preserved hepatic lobular architecture with more or less normal nearly as control. Hepatocytes (h) exhibit similar in many aspects to their corresponding control. An apparent increase of hepatocytes which appear binucleated (↑). Some portal tracts (P) appear nearly as control, except some areas contained large, dilated portal veins (v) with mononuclear cellular infiltration (l). Blood sinusoids (s) appear normally separating hepatocytes cords which are lined by endothelial (red arrow) and Kupffer cells (blue arrow). (H&E; ×10, ×20 and ×40)

Liver sections from the DMBA-treated group with CoQ10 (GIV) revealed a reduction in the DMBA-induced degenerative alterations. Hepatocytes appeared to be very similar to their control counterparts. The lobular structure of the liver was unaltered and the central veins were not obstructed. The hepatocytes appeared polyhedral cells with deeply acidophilic cytoplasm and vesicular nuclei with prominent nucleoli. An apparent increase of hepatocytes which appeared



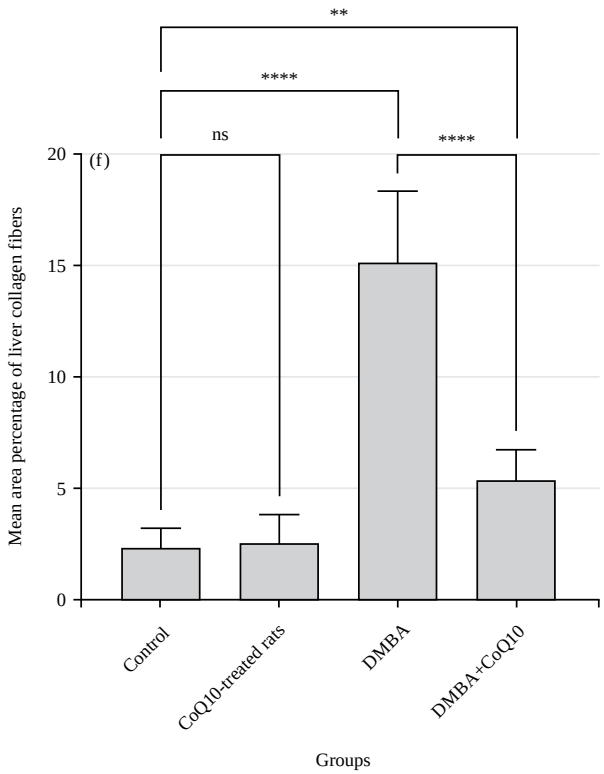


Fig. 6(a-f): Photomicrographs of sections of rat liver from, (a) Control group (GI), (b) CoQ10-treated rats (GII), (c-d) DMBA group (GIII), (e) DMBA+CoQ10 group (GIV) and (f) Mean±Standard Deviation (SD)

(a) Show normal distribution of collagen fibers (↑) around central vein (CV), trabecular septa (S), in-between hepatocytes (h) and in the portal area (P). (b) Show no histological difference from control group GI. (c-d) Show marked increase in collagen fibers. (e) Show moderate collagen fibers deposition. (f) Show Mean±Standard Deviation (SD) of the area percentage of the collagen fibers for the control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+Co Q10-treated rats (G IV) groups (n =10 rat/group). ns: Non-significant, Significant at **p<0.01 and ****p<0.0001. Mallory trichrome stain; ×10

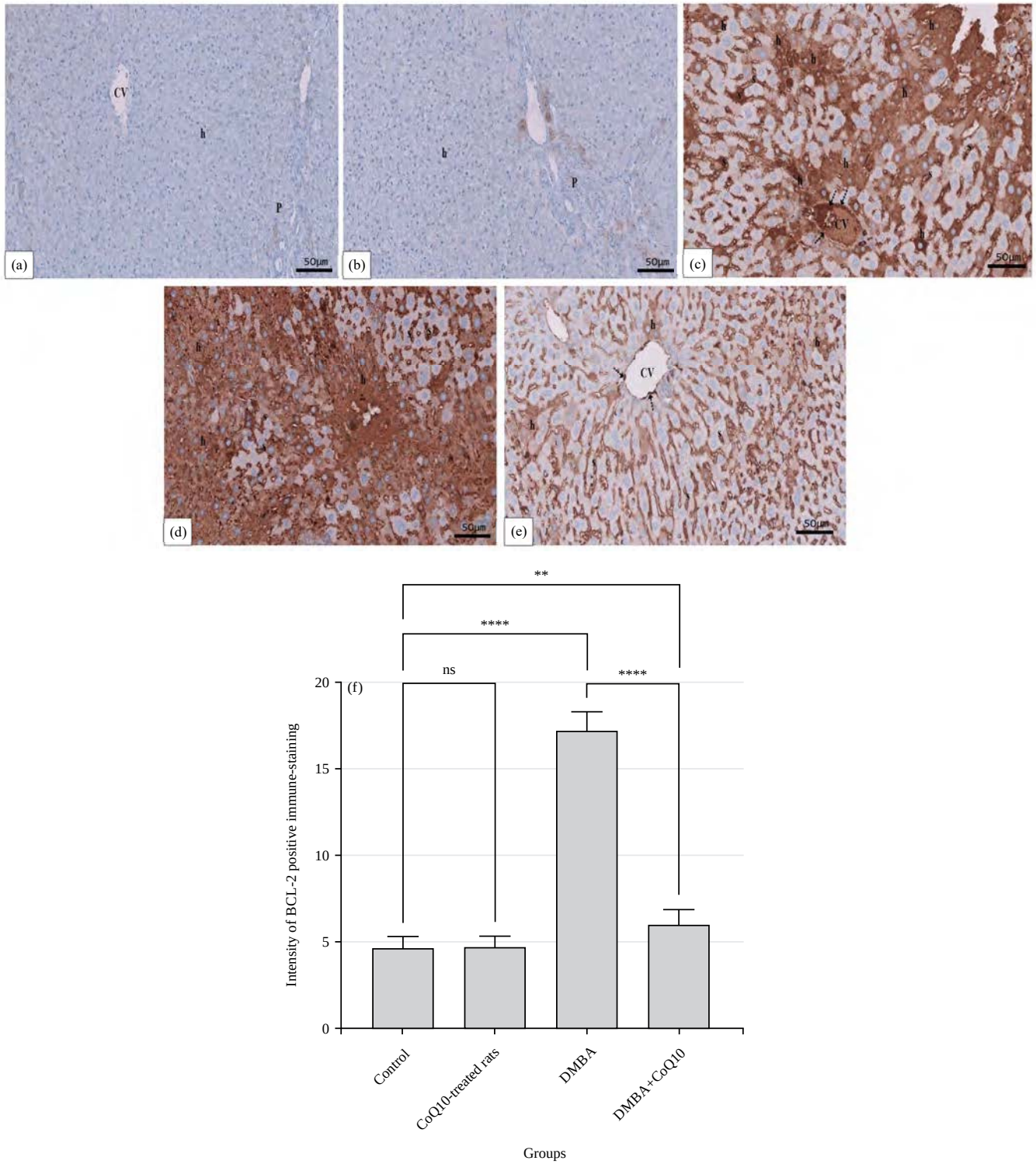


Fig. 7(a-f): Photomicrographs of sections of rat liver from, (a) Control group (GI), (b) CoQ10-treated rats (GII), (c-d) DMBA group (GIII), (e) DMBA+CoQ10 group (GIV) and (f) Mean±Standard Deviation (SD)

(a-b) show negative immunoexpression of BCL-2 in the cytoplasm of hepatocytes (h)and portal area (P). (c-d) DMBA group (GIII) show marked intense positive cytoplasmic immune reaction (↑) for Bcl-2 in the hepatocytes (h), surrounding central vein (CV) and cells lining the blood sinusoids (s). (e) Show moderate week positive cytoplasmic immune reaction (↑) for Bcl-2 in the hepatocytes (h), surrounding central vein (CV) and cells lining the blood sinusoids (s). (f) Mean±Standard Deviation (SD) of the intensity of BCL-2 positive immune-staining for the Control group (GI), CoQ10-treated rats (GII), DMBA rats (GIII) and DMBA+Co Q10-treated rats (GIV) groups (n = 10 rat/group), ns: Non-significant, Significant at **p<0.01, ****p<0.0001. BCL-2 Immunohistochemical stain ×20

binucleated. Hepatic arteries and bile ducts in some portal tracts were unaffected, but in other regions, massive, dilated portal veins with mononuclear cellular infiltration were present. Blood sinusoids appeared normally separating hepatocytes cords which are lined by endothelial and Kupffer cells (Fig. 5j-l).

Mallory trichrome stain: Mallory's trichrome sections revealed regular distribution of collagenous fibres in both the control (Fig. 6a) and CoQ10 (Fig. 6b) rat groups. Connective tissue septa were shown to contain a scant covering of corrugated collagenous fibres. The liver sections demonstrated very delicate meshwork of collagen fibers which were normally distributed around central veins, in-between hepatocytes and at the portal area. Collagen fibre deposition was much higher in DMBA rats compared to controls. Connective tissue septa, the regions around central veins and the portal tract (including the proliferating bile ducts) all contain very thick corrugated bundles of collagen. Notice marked increased collagenous fibers in between hepatocytes (Fig. 6c-d). The deposition of collagenous fibres was relatively mild in the DMBA/CoQ10 group (IV) compared to the control group. A layer of dense, corrugated collagenous fibres made up this moderate-thickness layer. However, there was still a region of enhanced density near the portal and central vein (Fig. 6e). Morphometric analysis corroborated the findings on histology. The animals given DMBA had the highest mean, followed by those given DMBA and CoQ10. The rats treated with CoQ10 alone or as a control group had the lowest results. As shown in Fig. 6f, the DMBA group exhibited a significant ($p<0.0001$) increase in mean area percentage of collagenous fibers (15.25 ± 3.10) as compared to the control group (2.36 ± 0.815) and CoQ10 (2.59 ± 1.22) groups. Interestingly, after CoQ10 treatment, the rats achieved a significant ($p<0.0001$) decrease in the mean area percentage of collagen (5.37 ± 1.35) versus the DMBA group. In addition, there was a significant ($p=0.005$ and $p = 0.011$, respectively) increase in the mean area percentage of collagen in rats in DMBA+CoQ10 versus the control and CoQ10 groups.

Immunohistochemical stain: The liver section of control (Fig. 7a) and CoQ10 (Fig. 7b) rats groups showed negative immunoexpression of BCL-2 in the cytoplasm of hepatocytes. In contrast, DMBA rats showed marked intense positive cytoplasmic immune reaction for Bcl-2 in the hepatocytes, surrounding the central vein and cells lining the blood sinusoids (Fig. 7c-d). While sections of DMBA treated with CoQ10 group (IV), showed a noticeable moderate weak Bcl-2 immunoexpression in the cytoplasm, but it was still more than the control group (Fig. 7e). The immunohistochemical results were confirmed morphometrically. The animals given DMBA had the highest mean, followed by those given DMBA and CoQ10. The lowest value was noted in the control rats and rats treated with CoQ10 alone. As shown in Fig. 7f, DMBA group showed a significant ($p<0.0001$) increase in the intensity of BCL-2 positive immune-staining (17.30 ± 1.00) versus the control group (4.68 ± 0.60) and CoQ10 (4.76 ± 0.58) group. Interestingly, after CoQ10 treatment, the rats achieved a significant ($p<0.0001$) decrease in the intensity of BCL-2 positive immune-staining (6.06 ± 0.83) versus the DMBA group. In addition, there was a significant ($p = 0.001$ and $p = 0.003$, respectively) increase in the intensity of BCL-2 positive immune-staining in rats in DMBA+CoQ10 versus the control and CoQ10 groups.

DISCUSSION

Cancers of the breast, lung, lymphoid tissue, gut and skin are studied using rodent models where DMBA acts as a carcinogen and immunosuppressor to study mutation-inducing agents[18,31]. After DMBA is given, hepatocytes will take up the chemical. When xenobiotics undergo metabolism mediated by cytochrome P450, CYP1B1 and microsomal epoxide hydrolase, they are transformed into a proximal carcinogenic metabolite called DMBA-3,4-diol, which is known to cause harm to the liver. When these carcinogenic metabolites leave the liver, they travel through the bloodstream and interact with rapidly dividing cells, where they can cause DNA adducts and mutations that pave the way for cancer[13]. After DMBA is given, hepatocytes will take up the chemical. When xenobiotics undergo metabolism mediated by cytochrome P450, CYP1B1 and microsomal epoxide hydrolase, they are transformed into a proximal carcinogenic metabolite called DMBA-3,4-diol, which is known to cause harm to the liver. When these carcinogenic metabolites leave the liver, they travel through the bloodstream and interact with rapidly dividing cells, where they can cause DNA adducts and mutations that pave the way for cancer[1].

Body weight gain increase was significantly reduced in the present study among DMBA-treated rats. These results were in agreement with the findings reported previously[32,33]. Here we see that compared to DMBA-treated rats, CoQ10-treated animals gained significantly more weight after treatment. Possible explanation: CoQ10's potent antioxidant properties protect it from DMBA-induced oxidative stress[34-36].

Many disorders can be traced back to the presence of reactive oxygen species (ROS). Values for White Blood Cell (WBC), eosinophil, neutrophil and monocyte count in DMBA-exposed rats rise, as do those for oxygen radical generation and lipid peroxidation, which are linked to the etiology of many diseases and the hazardous effects of numerous substances[37]. Increased neutrophil counts are another sign of a hematological change associated with DMBA-induced liver problems. Liver disorders such as alcoholic liver disease, non-alcoholic fatty liver diseases, liver cirrhosis and fibrosis, viral hepatitis, liver failure and liver malignancy are associated with a high count of neutrophils because of the presence of bacteria or infection in addition to autocrine, paracrine and immune system regulating functions[37]. The vast majority of circulating leukocytes are neutrophils, which play important roles in a wide range of immunological responses and inflammatory events[38,39]. The experiment data of the current study were in line with Kabeel *et al.*[27] and El-Masry *et al.*[40].

According to these results, administration of CoQ10 restored body weight in chemically generated leukemic rats. In addition, when comparing the leukemic rats to the control rats, CoQ10 brought back normal levels for total white blood cell count, lymphocyte count and neutrophil count. In addition, the number of blasts in the leukemic rats was reduced by CoQ10. Consistent with prior research, current findings showed that CoQ10 has a cancer-preventing impact[34]. According to the results of this study's hematological profile, CoQ10 helps restore the normally functioning immune system in DMBA-exposed rats' livers.

Histological examination of liver tissue did not reveal any cancerous cells. Hepatic damage indicators (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) in the blood were also analyzed. Not only would the liver homogenate demonstrate DMBA-related injuries, but the numerous serum indicators will echo those findings. The aminotransferases ALT and AST are both found in the liver. Liver enzyme AST facilitates glutamate to aspartate reversible amino group transfer[41]. The ALT is another important enzyme that catalyzes the reversible transfer of an amino group from L-alanine to-ketoglutarate[42]. The ALP is a crucial serum measure because of its role in phosphate removal from a wide range of compounds[43]. Sinusoidal and bile duct membranes contain ALP, an essential blood serum for diagnosing liver disorders[44]. Both AST and ALT enzyme activity were highest in DMBA-treated rats. Serum elevation of these enzymes implies liver injury. The ALP enzyme showed the same outcomes. The liver injury may have caused the transaminases and ALP to leak out of the liver cells and into the blood, leading to an increase in the activity of these enzymes. Increased activity of liver marker enzymes was also observed when hepatic damage was produced by DMBA, as found by Singh *et al.*[10]. Increased AST, ALT and ALP values were reported in the DMBA group, suggesting hepatic damage in the study by Arora *et al.*[43]. Impaired hepatic excretion in liver parenchyma or ductal cells, as revealed by Silitonga *et al.*[37] is the cause of elevated ALP reading. However, the levels of liver enzymes were significantly reduced in the group of rats treated with CoQ10 (GIV). Serum elevation of these enzymes implies liver injury. Treatment with CoQ10 significantly reduced their levels, suggesting that CoQ10 may have a hepatoprotective effect.

Several pathologic states have been linked to ROS[45,46]. This study shows that DMBA administration significantly increased MDA levels while decreasing antioxidant indicators in rats, indicating oxidative stress (GSH and SOD). By boosting GSH and SOD activity and decreasing MDA, CoQ10 therapy counteracted oxidative stress. Since it is widely established that DMBA causes some of its harmful effects on DNA by inducing the creation of carcinogenic free radicals, the oxidative activity of CoQ10 would amplify the effect of DMBA, speeding up carcinogenesis in DMBA-exposed rats[9]. The antioxidant glutathione protects cells from free radicals, peroxides and other noxious chemicals by acting as a reducing agent. Cellular levels of vitamin C and E are maintained in their active forms by reduced Glutathione (GSH). The immune system is boosted, free radicals are mopped up and the metabolism of carcinogens is altered thanks to these vitamins. The GSH levels were observed to be lower in the DMBA group compared to the control group. Possible explanations for the reduced levels of these antioxidants in DMBA-induced mice include their extensive use in mitigating the enormous free radicals generated by this situation[47]. A sign of oxidative cell damage, lipid peroxidation is a chain reaction involving the oxidation of polyunsaturated fatty acids in membranes and is triggered by ROS. The MDA is a by-product of lipid peroxidation and is measured to get an idea of how quickly chain reactions are happening in a certain tissue. The DNA damage may be caused by lipid peroxidation by-products, it has been reported. The rate of lipid peroxidation in cells is thought to correlate negatively with its concentration; in other words, as lipid peroxidation increases, cell division slows[34]. We found that MDA levels were greater in DMBA-treated rats, which may be attributable to the production of free radicals because of the depletion of antioxidants and subsequent oxidative stress.

Damaged hepatocytes in DMBA-treated rats were characterized histologically by severe cytoplasmic vacuolization, high eosinophilic cytoplasm, necrosis, compaction of blood sinusoids and hyperchromatic nuclei. Abdelghffar *et al.*[48] found that hepatocellular enlargement caused a loss of hepatic cord regularity in liver slices from DMBA-treated mice, therefore these changes are consistent with their findings. Some hepatocytes in the pericentral regions had vacuolated cytoplasm, but otherwise, blood sinusoids were no longer apparent. Necrotic alterations of hepatocytes in periportal areas also revealed that liver lesions reduce the total number of hepatocytes, inhibit the uptake of indirect bilirubin from the plasma and disturb the transport mechanism of direct bilirubin through the bile ducts[49]. Hepatocyte cytoplasm was found to include vacuoles of varying sizes, as described by Thoolen *et al.*[50], which may promote membrane permeability. The build-up of lipids within the cytoplasm and the proliferation of pleomorphic mitochondria with a thick matrix may follow. Perhaps this could explain why there are so many eosinophils in the cytoplasm. The DMBA-administrated rats revealed an apparent increase of altered Kupffer cells with a large nucleus, similar observations were reported[32,51]. The ROS and oxidative stress are intermediary variables in the development of fibrosis and the activation of hepatic stellate cells (HSCs)[52,53]. This could explain the significant increase of collagen fibers in Mallory trichrome stain and the positive BCL-2 IHCs brownish reaction in blood sinusoids.

Group IV revealed preservation of the histological structure of the hepatic tissue with apparently normal blood sinusoid and central veins. The CoQ10 is a rich source of antioxidants. They prevent lipid peroxidation by acting as free radicals scavengers. The CoQ10 also has anti-inflammatory properties. Treatment with CoQ10 demonstrates a decrease in hepatic vacuoles, inflammation, congestion and fibrosis. All these findings link with the hepatoprotective, antioxidant and anti-inflammatory properties of CoQ10[54]. Since its central role in mitochondrial bioenergetics has been established by previous research, CoQ10 has attracted a lot of attention as a dietary supplement[55]. Among several biological actions, the hepatoprotective role of CoQ10 shows a remarkable effect, susceptible to counteract many metabolic problems associated with DMBA toxicity. Specifically, Group IV, which received CoQ10, demonstrated a statistically significant decrease in the mean area percent of collagen fibers and BCL-2 IHCs reaction in hepatocytes and inside blood sinusoids as compared to group III, which did not. This was documented by Lamia *et al.*[28], who reported the hepatoprotective effect of CoQ10 against inflammation and fibrosis by CoQ10 impedes Transforming Growth Factor-β1 (TGF-β1) expression in hepatic stellate cells to inhibit fibrosis leading to hepatic damage.

CONCLUSION

This study highlights that the treatment with CoQ10 attenuated the alleviation of leukemia in rats that were induced by DMBA. In addition, we found that CoQ10 significantly decreased the levels of liver enzymes, oxidative stress indicators and degenerative liver alterations, claiming that it has strong hepatoprotective effect. In conclusion, our findings suggest that CoQ10 intervention is a potentially effective therapy for leukaemia and liver impairment caused by leukaemia.

SIGNIFICANCE STATEMENT

Coenzyme Q10 (CoQ10) is a cofactor in the mitochondrial electron transport chain that benefits liver disorders. Polycyclic aromatic hydrocarbon (PAH) 7,12-dimethylbenz[a]-anthracene (DMBA) promotes carcinogenesis. This study investigated the potential anti-leukemic effects of CoQ10 in a DMBA-induced leukemic rat model. The CoQ10 treatment significantly reduced liver enzyme levels relative to leukemia-model animals. Malondialdehyde (MDA) levels were much lower in CoQ10 group compared to the DMBA group, whereas Glutathione (GSH) and superoxide dismutase (SOD) levels were significantly increased. Histopathological examination of rat DMBA livers revealed a loss of normal hepatic architecture, which was restored after administration of CoQ10. Treatment with CoQ10 attenuated the alleviation of DMBA-induced leukemia in rats, claiming its potential as an effective therapy for leukaemia and liver impairment caused by leukaemia.

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