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RESEARCH

Vitamin B_{12} and coenzyme Q_{10} ameliorated alcohol-driven impairment of hematological parameters, inflammation, and organ damage in a mouse model

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Abstract

Introduction Chronic alcohol consumption is associated with a myriad of negative physiological and biochemical changes in humans. Vitamin B12 and coenzyme Q10 (CoQ10) are novel antioxidants and anti-inflammatory agents.

Purpose The objective of this study was to determine the impact of oral supplementation with vitamin B12 and CoQ10, in attenuating deleterious effects associated with alcohol exposure in Swiss albino mice.

Methods Group one was normal control, the second group received 5 g/kg alcohol; the third group received 6 mg/kg b/w of vitamin B12 and 5 g/kg alcohol; the fourth group received 6 mg/kg b/w of vitamin B12, 200 mg/kg b/w CoQ10, and 5 g/kg alcohol, the fifth group 200 mg/kg b/w of CoQ10 and 5 g/kg alcohol.

Results Oral administration of vitamin B12 and CoQ10 alone or in combination significantly ameliorated alcohol-induced impairment of hematological parameters and stabilized alcohol-induced alteration of the lipid profile. Notably, administration of either vitamin B12 or CoQ10 significantly blocked alcohol-induced depletion of reduced glutathione levels. Furthermore, vitamin B12 and CoQ10 stabilized the levels of pro-inflammatory cytokines (TNF-α and IFN-γ) when administered alone or in combination. Remarkably, the administration of CoQ10 and vitamin B12 significantly attenuated alcohol-induced liver and kidney inflammation and pathology.

Conclusion Administration of either vitamin B12 or CoQ10 alone or in combination can protect from the toxic effects of chronic alcohol exposure.

Keywords Vitamin B12 · Coenzyme Q_{10} · Alcohol toxicity · Inflammation · Oxidative stress

Introduction

Heavy consumption and abuse of alcohol contribute to 3 million deaths every year [1]. Excessive alcohol intake is associated with intestinal injury, alcohol liver disease, neurological impairment, diabetes mellitus, and carcinogenesis [2]. Indeed, alcohol liver disease continues to attract more attention throughout the world due to its high morbidity and mortality rate [1]. Liver damage can progress to liver cirrhosis and liver cancer. From existing research, it is evidently clear that chronic alcohol use has far-reaching consequences, devastating vital physiological and biochemical processes.

Additionally, previous data have demonstrated that alcohol administration induces the production of reactive oxygen species (ROS), including superoxide anion, hydrogen peroxide, and hydroxyl radical [3]. Many studies have shown that both oxidative stress and inflammation play a vital biological

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role in the pathogenesis of the alcoholic liver disease [2]. Severe oxidative stress is often characterized by the depletion of endogenous glutathione, and the antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase [4]. Alcohol-induced elevation of ROS has been shown to damage cellular components such as proteins, lipids, and nucleic acids. A study by Hernández et al. [5] demonstrated alcohol-related oxidative stress–induced neurotoxicity and neurodegeneration. Reduced glutathione is known to scavenge free radical species and generates α-tocopherol that maintains protein sulfhydryl groups [6].

The proliferation of the pro-inflammatory cytokine tumor necrosis factor-α (TNF-α) is associated with alcoholic liver disease (ALD) [7]. Earlier studies have shown that alcohol-fed mice produce higher levels of TNF-α [8]. Moreover, alcohol administration in mice has been found to increase gut permeability that allows the translocation of bacterial-derived molecules, especially lipopolysaccharide (LPS), that in turn activates liver cells (Kuppfer cells) to produce the pro-inflammatory cytokines (IL-6, TNF-α, IFNγ) which cause inflammation and liver injury [9]. Tracer studies in animals and humans have demonstrated that alcohol intake can alter liver and serum lipid concentration [10].

Currently, silymarin which is an extract from milk thistle *Silibum marianum*, contains a bioactive flavonoid that is used to treat alcohol-induced liver damage as well as liver cirrhosis [7, 11]. In vivo and in vitro studies have demonstrated that silymarin has the capacity to modulate cytokine production and is mainly active towards hydroxyl radicals and hypochlorite with no quenching effects on other ROS molecules [7]. The major limitation of silymarin is its ability to cause gastrointestinal injury and allergic reactions [7]. To date, there is no pharmacological or alternative intervention to prevent alcohol-related injury to the liver once exposure has occurred [12, 13]. The prevention of the harmful effects linked to alcohol remains the cardinal challenge, compounded with a lack of effective and safe drugs for the management of alcohol toxicity. Consequently, the search for new alternative strategies for the management of alcohol toxicity is of great importance. Additionally, no medication in the market is prescribed for individuals who are still drinking alcohol but is only available for people who have ceased drinking or trying to maintain abstinence. Due to alcohol-driven derangement of oxidative balance and inflammation, it is possible that interventions that boost anti-oxidant capacity and stabilize inflammation could benefit chronic alcohol users.

The present study sought to determine the capacity of oral supplementation of vitamin B_{12} and CoQ_{10}, novel antioxidants, and anti-inflammatory agents, to attenuate deleterious effects associated with alcohol exposure. Coenzyme Q_{10} also referred to as ubidecarenone is a potent antioxidant and anti-inflammatory molecule, which has been shown to regulate cytokine production and is able to attenuate oxidative stress [14]. CoQ_{10} has been reported to inhibit the production of IL-6, TNF-α, and NF-κB pathway activation [15]. A recent study has demonstrated the ability of CoQ_{10} to protect from oxidative stress and neuro-inflammation during experimental cerebral malaria (ECM) [15]. However, the application of CoQ_{10} and vitamin B_{12} as a protection strategy against alcohol-induced toxicity has not been investigated.

Vitamin B_{12} (cobalamin) is a water-soluble vitamin that also possesses antioxidant and anti-inflammatory activities. Current studies have shown that it has a positive modulatory potential against oxidative stress and inflammation [16, 17]. Vitamin B_{12} modulates inflammatory response by downregulating the transcription factor NF-kB pathway [18]. The choice of compounds with proven antioxidant and anti-inflammatory activity constitutes a relevant strategy to counter alcohol toxicity. Findings from this study demonstrate clearly that vitamin B_{12} and CoQ_{10}, individually or when combined, actively ameliorate alcohol-induced hematological alteration, oxidative stress, organ pathology, and inflammation. These new findings demonstrate the importance of these anti-oxidants as a novel treatment strategy against alcohol toxicity that can be used alongside existing pharmacological therapy to improve treatment outcomes.

Materials and methods

Experimental animals

This study utilized male Swiss albino mice aged about 6 weeks, under strict consideration of the ARRIVE checklist for reporting animal research [19]. To ensure the results of the study are free from parasitic effects, mice were de-wormed with Ivermectin (Anupco Suffolk England) subcutaneously and left to acclimatize for 1 week before the start of the experiment to normalize confounding physiological changes and to restore homeostasis following transportation [20]. The mice were housed in the animal facility with a controlled temperature (21–25 °C) and 12 h of light and dark cycle with free access to mice pellet and water ad libitum. Wood chippings were used as bedding material.

Experimental design

Swiss albino mice were randomly divided into five treatment groups ($n = 10$) (Table 1). Group I mice were orally administered with distilled water (vehicle). Group II mice were treated with 5 g/kg alcohol. Group III mice received 5 g/kg alcohol and vitamin B_{12} (6 mg/kg). Group IV mice were treated with 5 g/kg alcohol and CoQ_{10} (200 mg/kg). Group V mice were orally administered with 5 g/kg alcohol, vitamin B_{12} (6 mg/kg), and CoQ_{10} (200 mg/kg). All treatments were done orally daily for 45 days by the use of gavage.

Table 1 Experimental design

Groupings	Treatment	Duration
Group 1 (normal control group)	Distilled water	45 days
Group 2 (negative control)	5 g/kg alcohol	45 days
Group 3 (experimental group)	5 g/kg alcohol + 6 mg/kg vitamin B_{12}	45 days
Group 4 (experimental group)	5 g/kg alcohol + 200 mg/kg CoQ_{10}	45 days
Group 5 (experimental group)	5 g/kg alcohol + 6 mg/kg vitamin B12 + 200 mg/kg CoQ_{10}	45 days

Preparation of ethyl alcohol, CoQ_{10} and Vitamin B_{12}

Ethyl alcohol (35% v/v) was prepared from absolute ethyl alcohol (99%) purchased from Sigma-Aldrich (St. Louis, MO, USA). A 35% (v/v) preparation of ethyl alcohol was prepared by dilution with distilled water. Thirty-five percent ethyl alcohol at a dose of 5 g/kg was orally administered to mice. The ethyl alcohol dose was informed by previous findings that 35% v/v at the dose of 5 mg/kg induces alcohol liver injury in mice [13]. CoQ_{10} solution (Now Foods, Bloomingdale, IL, USA) was prepared by dissolving the powder supplement in 35% ethyl alcohol. CoQ_{10} concentrations were always freshly prepared and were covered with aluminum foil to protect them from the light before administration to the mice. A dose of 200 mg/kg was used for CoQ_{10} treatment in this study. The choice of dosage was based on previous findings that 200 mg/kg enhances neuroprotection [21]. In addition, this dose has been shown to have protection against oxidative stress and inflammation-induced pathology [15, 22–24]. Vitamin B_{12} solution (St. Louis, MO, USA) was prepared by dissolving the powder supplement in 35% ethyl alcohol. Vitamin B_{12} concentrations were always freshly prepared in the safety cabinet and were immediately covered with aluminum foil to protect from the light. A dose of 6 mg/kg was used for vitamin B_{12} treatment in this study based on previous findings that 6 mg/kg enhances protection against xenobiotic-induced toxicity [16].

Animal treatment

Oral administration of water, alcohol, and drugs was done daily for 45 days using a gastric oral gavage needle to the six experimental groups. Vitamin B_{12} and CoQ_{10} were freshly prepared and given 2 h before sunset when alcohol receptors were deemed to be active.

Determination of body weight gain

The body weight of each mouse was determined once a week for the entire experimental period. The weights of the kidney, heart, brain, spleen, and liver, kidney were measured at the end of the experiment after the euthanization of mice, followed by calculating the relative organ weights. General body and organ weights were measured using an analytical electronic balance (Mettler PM34, DoltaRange®).

Organ harvesting

Mice were euthanized with ketamine (50 mg/ml) after 45 days post-treatment. Blood samples were either collected in EDTA tubes (for hemogram analysis) or in plain red-top tubes containing no anticoagulants (for serum) through cardiac puncture. Liver, kidney, heart, spleen, and brain samples were also collected in snap-frozen cryo-vial tubes for GSH analysis. Organs for standard histology were fixed using 4% formalin and stored at room temperature for processing.

Determination of hematological value

Blood was collected directly from the heart during the euthanization of the mice in a 1-ml syringe and transferred to EDTA tubes. The blood samples were analyzed using an automated Bechman Coulter Counter (Coulter A-T diff TM) giving results for a complete blood count.

Sample preparation

Snap-frozen whole isolated organs were homogenized on ice (4°) in 0.5 ml of 0.25 M sucrose, 5 mM Hepes-Tris, and pH 7.4, with protease cocktail inhibitor to a final concentration of 10% (w/v). The homogenates were aliquoted into 0.5 microfuge tubes (to avoid an excessive freeze–thaw process) and stored in liquid nitrogen for analysis.

Reduced glutathione (GSH) assay

The total GSH content was determined by employing the method of Rahman et al. [25]. Briefly, the liver, spleen, lungs, heart, kidney, and brain homogenates were mixed with a solution containing sulphosalicylic acid (4.31% (w/v)) and 0.25 mM EDTA. The GSH in the homogenates were then determined chemically by reacting the GSH therein with Ellman's reagent (DTNB) and measuring the absorbance of the reaction product at 412 nm using a multi-detection microplate reader (Bio-Tek Synergy HT).

Biochemical analysis

At 45-day post-treatment, mice were bled through cardiac puncture after euthanization, and blood was collected into a plain red-top tube containing no anticoagulants. Then the blood was allowed to clot at room temperature for 2 h and then centrifuged at 10,000 × g at 4 °C for 5 min to obtain serum (Centurion Scientific Ltd K240R, UK). The serum was transferred into Eppendorf tubes for the analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), bilirubin (BIL), cholesterol (CHOL), high-density lipoproteins (HDL), and triglyceride (TRIG).

Histopathological examination of liver, kidney, and brain sample

The liver, kidney, and brain were harvested after perfusion phosphate-buffered saline and then fixed with 10% formaldehyde. Gradient dehydration was done in ascending concentrations of 50%, 70%, 90%, 95%, and 100% alcohol then embedded in paraffin wax in the automatic tissue processor. Sections of 5 µm thickness were cut in HM 310 rotary microtome and mounted on Mayer's egg albumin-coated glass slides. Each section was dewaxed in two changes of xylene for 2 min then rehydrated through descending grades of alcohol for thirty minutes (100%, 95%, 90%, 70%, and 50%) and further washed in tap water. The gradual removal of water from the tissues was done to avoid sudden shrinkage of the cells and secondly to avoid the rupture of the cells. The sections were stained with hematoxylin and then stained with eosin (H&E) (1% for 2 min). The sections were further dehydrated in ascending grades of alcohol for thirty minutes (70%, 80%, 95%, and 100%), cleared in three changes of xylene and mounted in DPX, and examined microscopically.

Cytokine ELISA

To measure tumor necrosis alpha (TNF-α), interferon-gamma (IFN-γ), or interleukin-10 (IL-10) from murine serum, kits from Thermo Fisher Scientific (Vienna, Austria) were employed. Sandwich ELISAs were performed in accordance with the manufacturer's protocol. The substrate solution utilized was tetramethylbenzidine (TMB) because the secondary antibodies were horse radish peroxidase conjugated, and the reaction was stopped by the addition of a few drops of 2N H_2SO4. The measurement of the optical density was done using an optical reader (Multiskan ex-355, Thermo Electron Corporation, Waltham, MA, USA) at 450 nm wavelength. The protein cytokine concentration was interpolated from the cytokine-protein standard curve.

Statistical analysis

Results are expressed as ± SEM. Differences among multiple groups were analyzed by one-way ANOVA followed by Tukey's post-hoc test. The significance between the groups was defined as $p < 0.05$. Statistical analysis was done using the GraphPad Prism software package (version 5.0).

Results

Vitamin B_{12} and CoQ_{10} did not have any effect on alcohol-induced general change on body weight

Exposure of alcohol to mice resulted in a significant decrease ($p < 0.05$) in weight when compared to the normal control (Fig. 1). An increase in weight was observed in mice administered with CoQ_{10} upon exposure to alcohol. However, the group of mice administered with alcohol and vitamin B12 or in combination with CoQ_{10} and vitamin B_{12} had a significant ($p < 0.05$) decrease in weight when compared to the normal control.

The effect of alcohol, vitamin B_{12} and CoQ_{10} on the weight of the brain, liver, kidney, and spleen

The weight of the brain and kidney was unaffected by alcohol exposure (Fig. 2A). Mice orally administered with alcohol registered a significant increase ($p < 0.05$) in liver and spleen weight (Fig. 2C and D). Administration of vitamin B_{12} and CoQ_{10} alone or in combination prevented alcohol-driven induction of weight increase for the liver and spleen.

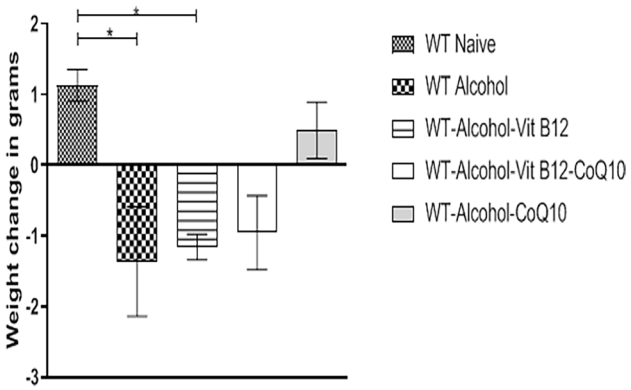

Fig. 1 The effects of alcohol, vitamin B_{12}, and CoQ_{10} on general body weight change. Comparison between various groups was done using one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$). Bars represent mean ± SEM

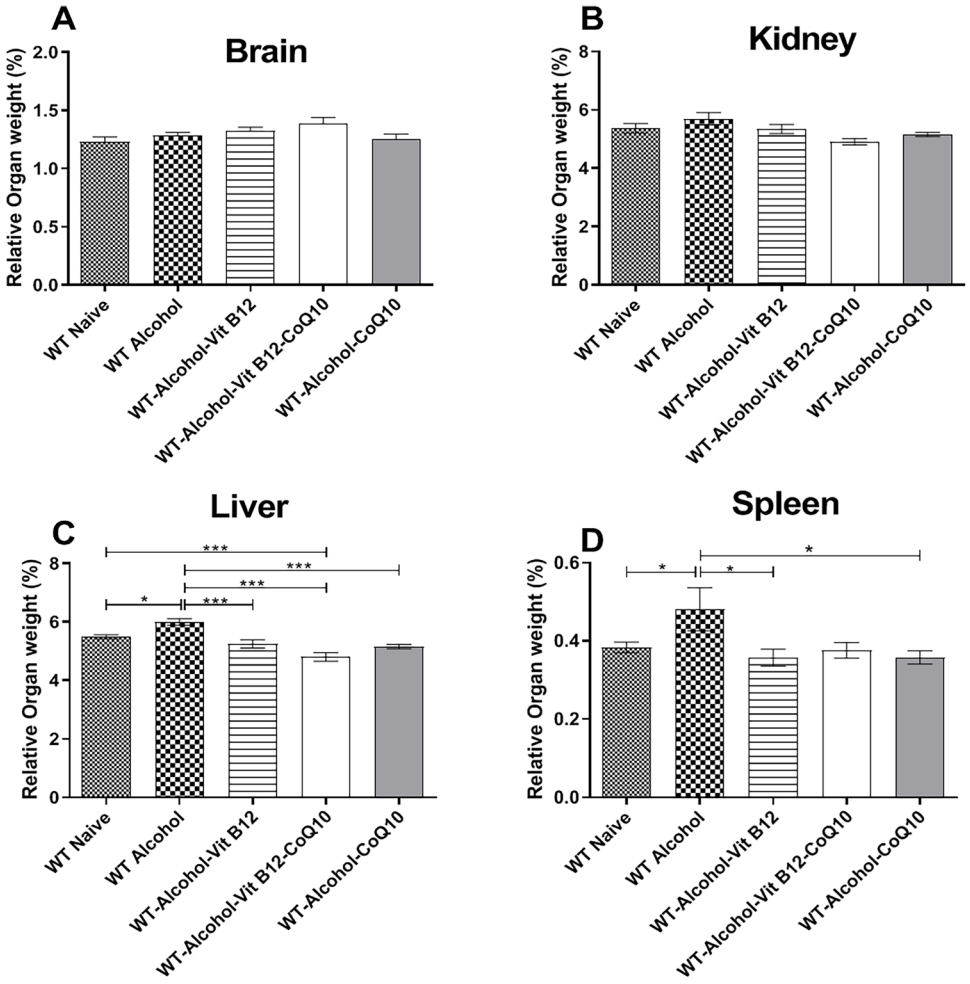

Fig. 2 Effects of vitamin B_{12} and CoQ_{10} on the relative organ weight following alcohol-induced toxicity in Swiss albino mice. Relative organ weight of the brain (**A**), kidney (**B**), liver (**C**), and spleen (**D**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$; $***p \leq 0.0001$). Bars represent mean ± SEM

Oral administration of vitamin B_{12} and CoQ_{10} prevented alcohol-driven depletion of hematocrit levels, red blood cells, hemoglobin, and mean corpuscular hemoglobin

Exposure to alcohol resulted in a significant decrease ($p < 0.05$) in the hematocrit levels (Fig. 3A). In the presence of vitamin B_{12} and CoQ_{10}, the alcohol-induced suppression of the hematocrit was ameliorated. Alcohol resulted in a significant decrease in the mean levels of RBCs (Fig. 3B) which was also accompanied by significantly low levels of Hb (Fig. 3B), indicative of anemia condition. Supplementation with either vitamin B_{12} or CoQ_{10} alone or in combination significantly restored the levels of RBC and Hb. Scrutiny of RBC indices: mean cell hemoglobin concentration (MCHC), mean cell (or corpuscular) volume (MCV), mean cell hemoglobin (MCH), red cell distribution width-standard deviation (RDW-SD), and red cell distribution width-coefficient variation (RDW-CV) were analyzed to determine the type of anemia induced by alcohol ingestion. Of all these RBC indices, only the MCH was significantly downregulated by alcohol and restored in the presence of vitamin B_{12} and CoQ_{10}. The rest remained unchanged (Fig. 3C–G).

Vitamin B_{12} and CoQ_{10} administration suppressed alcohol-induced leukocytosis

There was a significant elevation of white blood cells (WBC) in an alcohol-exposed group of mice (Fig. 4A). Administration of vitamin B_{12} and CoQ_{10} alone or in combination significantly stabilized WBC levels nullifying alcohol-induced elevation. In stark contrast, the mean levels of WBC subtypes that include neutrophils, lymphocytes, monocytes, eosinophils, and basophils were not significantly changed across all the treatment groups (Fig. 4B–F).

Treatment with alcohol in the presence of vitamin B_{12} and CoQ_{10} did not alter platelet levels

None of the treatments significantly altered platelets or their indices platelet distribution width (PDW), mean platelet volume (MPV), and platelet-large cell ratio (P-LCR) (Fig. 5A–D).

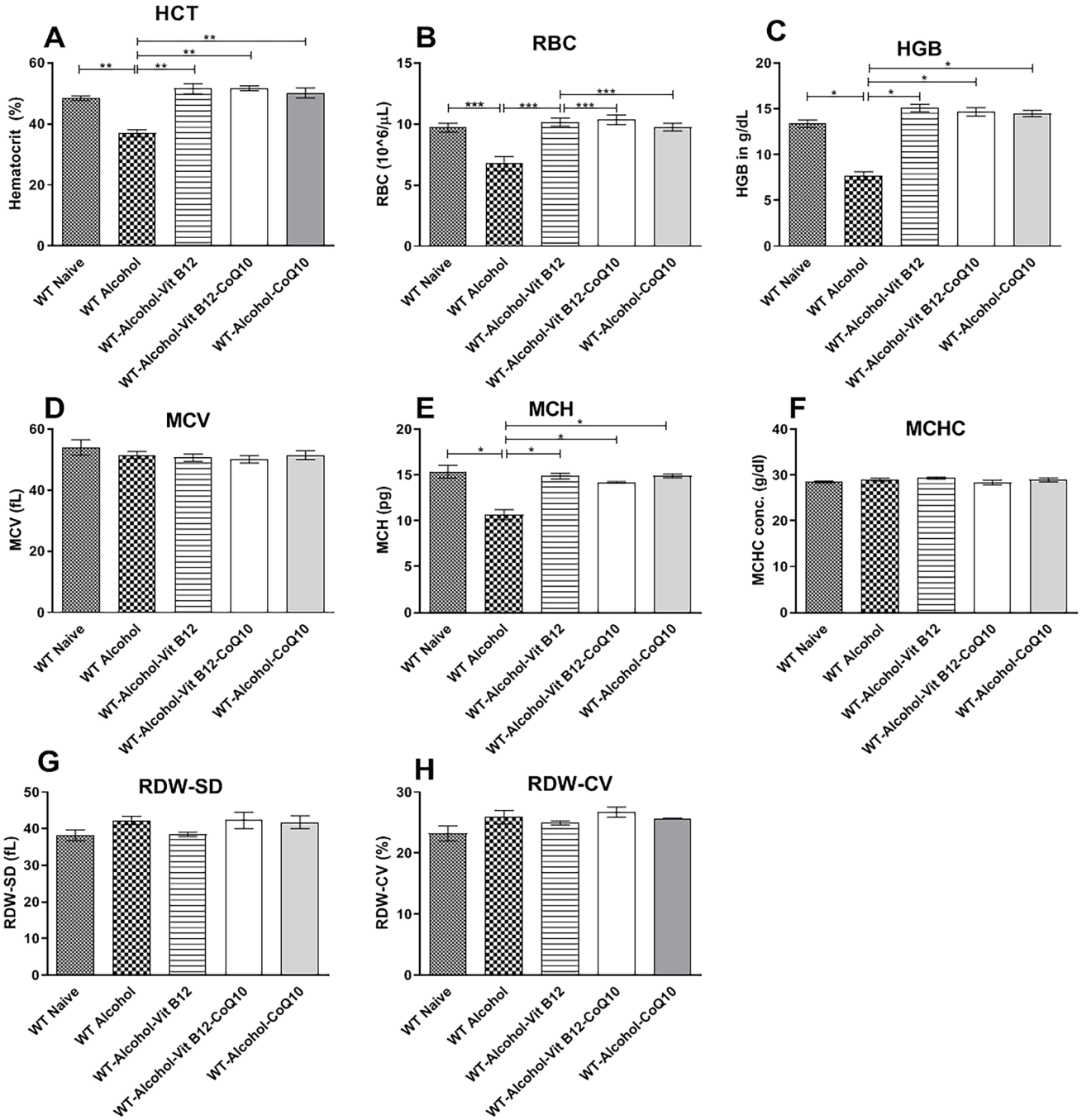

Fig. 3 The effect of alcohol, vitamin B_{12}, and CoQ_{10} on various red blood cell parameters and indices. Mean number of hematocrit (**A**), RBC (**B**), HGB (**C**), MCV (**D**), MCH (**F**), MCHC (**F**), RDW-SD (**G**), and RDW-CV (**H**) from the blood of male Swish albino mice. Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$; $***p \leq 0.0001$). Bars represent mean ± SEM

Vitamin B_{12} and CoQ_{10} supplementation attenuated alcohol-induced depletion of glutathione

The current study sought to determine the levels of cellular GSH in the kidney, spleen, lungs, brain, liver, and heart tissues in mice administered with alcohol, vitamin B_{12} and CoQ_{10} individually or when combined. In the alcohol-supplemented group, there was an observable reduction in mean cellular levels of GSH in the kidney ($p < 0.001$; Fig. 6A), and a significant reduction in the mean cellular levels of GSH in the spleen ($p < 0.01$) (Fig. 6B), lungs ($p < 0.001$) (Fig. 6C), brain ($p < 0.001$) (Fig. 6D), and liver ($p < 0.001$) (Fig. 6E).

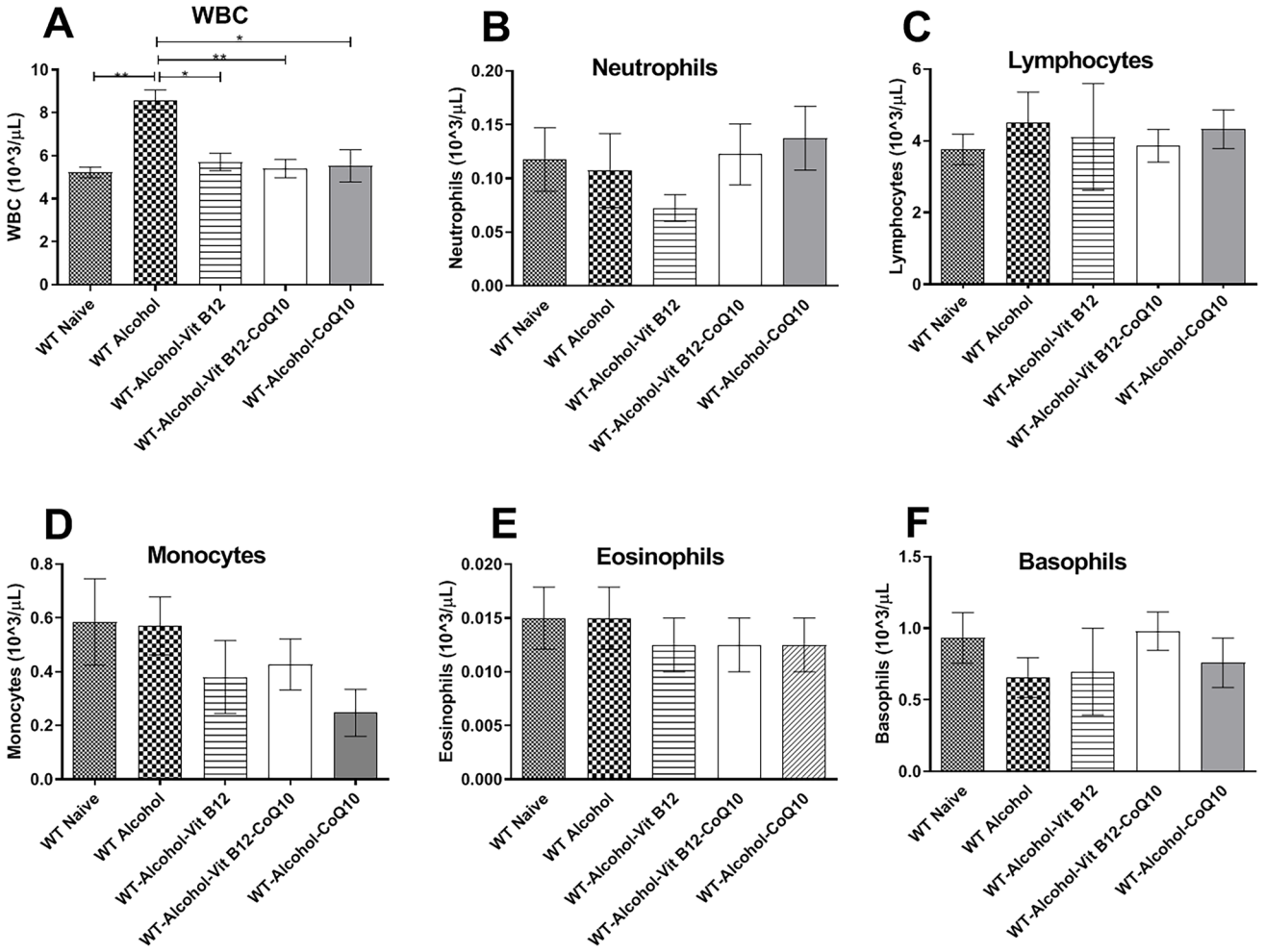

Fig. 4 Effects of vitamin B_{12} and CoQ_{10} on white blood cells and sub-types following alcohol-induced toxicity in Swiss albino mice. Blood levels of WBC (**A**), neutrophils (**B**), lymphocytes (**C**), monocytes (**D**), eosinophils (**E**), and basophils (**E**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$; $**p \leq 0.001$). Bars represent mean ± SEM

Notably, supplementation with either CoQ_{10} or vitamin B_{12} or in combination significantly prevented alcohol-induced depletion of cellular GSH levels in these organs. The level of GSH in the heart tissue was comparable across all the treatment groups (Fig. 6F).

The impact of alcohol, vitamin B_{12} and CoQ_{10} on cytokines

Cytokines play a pivotal role in the modulation or aggravation of the inflammatory processes during the induction of adaptive immune responses. In the current study, the levels of the pro-inflammatory cytokine tumor necrotic factor-alpha (TNF-α) and interferon-gamma (IFN-γ), as well as the anti-inflammatory cytokine interleukin-10 (IL-10), were assessed. In association with the presence of active oxidative stress, IFN-γ and TNF-α levels were significantly increased in the serum of alcohol-exposed mice on day 45 post-treatment ($p < 0.05$) (Fig. 7A–B). A significant decrease in the levels of the anti-inflammatory cytokine IL-10 was observed for the group administered with alcohol ($p < 0.05$) (Fig. 7C). In the presence of vitamin B_{12} and CoQ_{10}, such induction of alcohol-induced elevation of serum levels of TNF-α and IFN-γ was abrogated while the levels of IL-10 were restored to baseline levels. The balance between the levels of pro-inflammatory and anti-inflammatory cytokines is important in determining the extent of inflammation. In this study, our analysis showed a markedly net imbalance in TNF-α: IL-10 and IFN-γ: IL-10 ratio in alcohol-exposed group of mice (Fig. 7D–E). Importantly, vitamin B_{12} and CoQ_{10} supplementation abrogated the alcohol-induced imbalance of these ratios, a clear indication of protection against the inflammatory process.

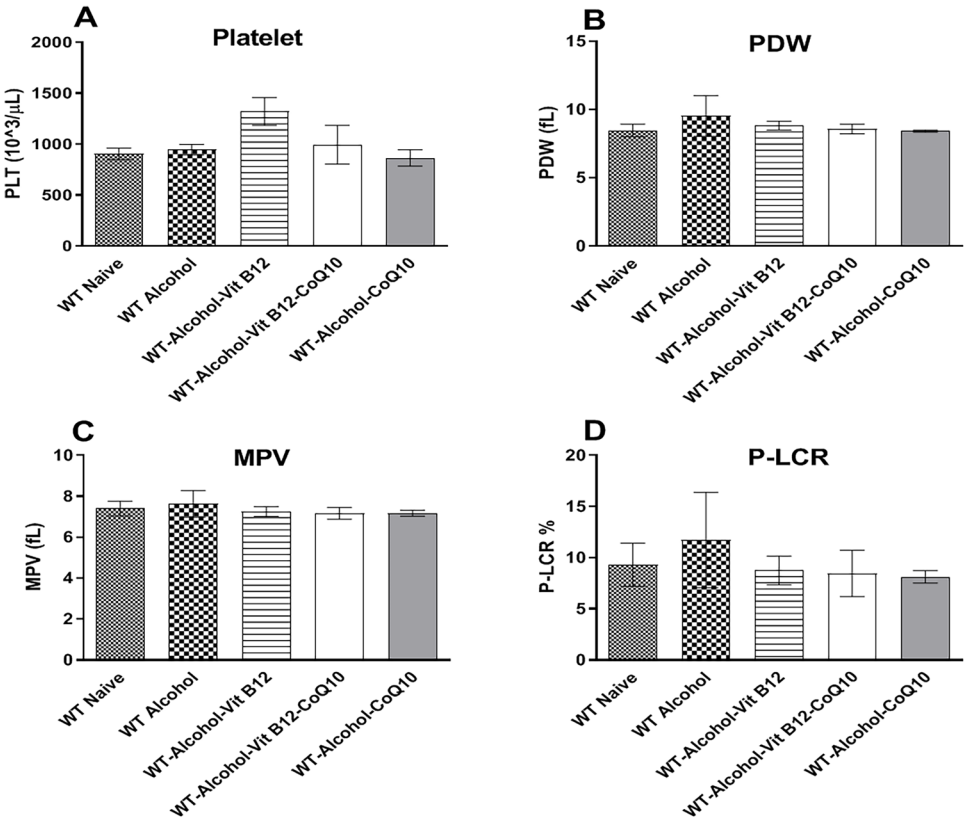

Fig. 5 The effect of alcohol on the platelets in the presence or absence of vitamin B_{12} and CoQ_{10}. Mean platelets (**A**), PDW (**B**), MPV (**C**), P-LCR (**D**) from the blood of male Swish albino mice. Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test. Bars represent mean ± SEM

Liver enzyme assays from mice administered with alcohol with or without vitaminB_{12} and CoQ_{10}

In order to determine whether supplementation with vitamin B_{12} and CoQ_{10} can attenuate hepatotoxicity induced by alcohol administration, we performed liver enzyme tests. Exposure to alcohol significantly increased the levels of alanine aminotransferase ALT ($p < 0.05$), aspartate aminotransferase AST ($p < 0.05$), and gamma-glutamyl transaminase GGT ($p < 0.05$) (Fig. 8A–C respectively), indicative of alcohol-induced hepatotoxicity. Supplementation with either CoQ_{10} or vitamin B_{12} significantly restored levels of AST and GGT ($p < 0.05$). Combined administration of vitamin B_{12} and CoQ_{10} significantly restored the levels of GGT ($p < 0.01$), a clear manifestation of a protective role by CoQ_{10}/vitamin B_{12} against alcohol-driven damage.

Vitamin B_{12} and CoQ_{10} supplementation nullified alcohol-induced elevation of bilirubin

A significant upsurge in the total bilirubin level was observed in the group exposed to alcohol ($p < 0.05$; Fig. 9A), whereas supplementation with vitamin B_{12} alone resulted in significantly reduced levels of bilirubin ($p < 0.01$; Fig. 9A) relative to an alcohol group. Meanwhile, the presence of alcohol, vitamin B_{12} and CoQ_{10} did not result in a statistical significance on the levels of serum direct bilirubin (Fig. 9B).

Supplementation with vitamin B_{12} or CoQ_{10} stabilized lipid levels

Significantly elevated levels of total cholesterol ($p < 0.05$), high-density lipoprotein (HDL) ($p < 0.05$), and triglycerides ($p < 0.05$) were observed in the serum of mice exposed to alcohol (Fig. 10A–C respectively). The group of mice supplemented with vitamin B_{12} or CoQ_{10} alone or in combination had normal levels of total cholesterol, HDL, and triglycerides.

Supplementation with vitamin B_{12} or CoQ_{10} nullified alcohol-driven elevation of serum creatinine

To determine if vitamin B_{12} or CoQ_{10} supplementation has ameliorative effects on alcohol-induced toxicity in the kidney, serum creatinine levels were analyzed. Creatinine levels were significantly elevated ($p < 0.05$) in mice exposed to alcohol (Fig. 11). Mice orally administered with either vitamin B_{12} or CoQ_{10} alone or in a combination demonstrated normal levels of creatinine in their serum.

Determination of the impact of alcohol on the cellular structure of the kidney tissue with or without vitamin B_{12} and CoQ_{10}

Histopathological changes in the kidneys are shown in Fig. 12. The control group showed normal kidney

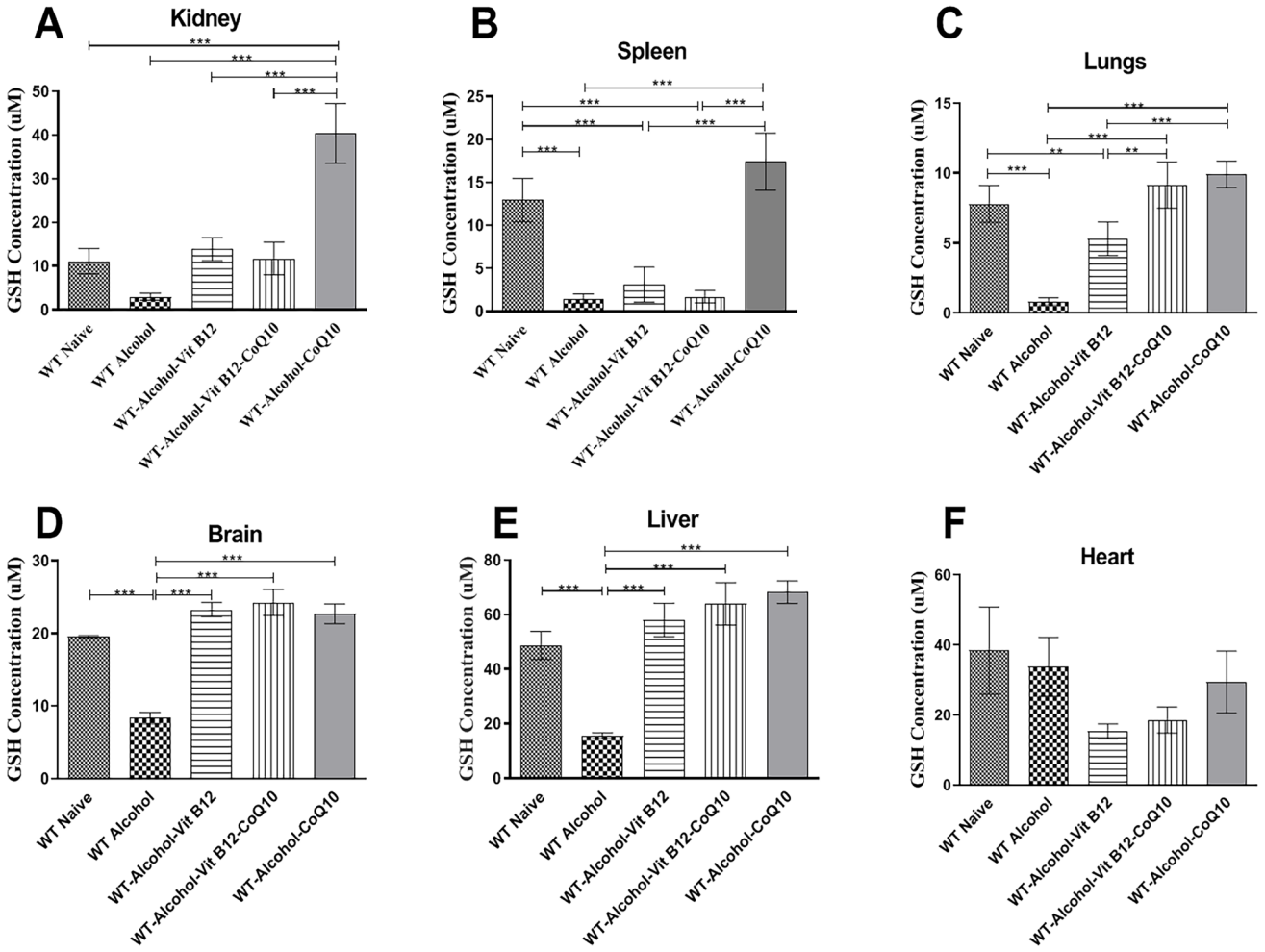

Fig. 6 Effects of vitamin B_{12} and CoQ_{10} on cellular GSH levels following alcohol-induced toxicity in Swiss albino mice. Cellular GSH levels of the kidney (**A**), spleen (**B**), lungs (**C**), brain (**D**), liver (**E**), and heart (**F**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $^{**}p \leq 0.001$ $^{***}p \leq 0.0001$). Bars represent mean ± SEM

histo-architecture (Fig. 12A). While mice administered with alcohol demonstrated histopathological changes, i.e., focal areas of tubular epithelial cell swelling (Fig. 12B). Notably, mice that were administered with vitamin B_{12} following alcohol exposure revealed mild focal areas of tubular epithelium degeneration and congestion of renal blood vessels (Fig. 12C). Furthermore, mice exposed to alcohol and treated with the combination of vitamin B_{12} and CoQ_{10} and those treated with CoQ_{10} alone had normal kidney architecture (Fig. 12D–E).

Vitamin B_{12} and CoQ_{10} ameliorated alcohol-induced blood vessel congestion in the liver

Histopathological changes in the liver tissue are depicted in Fig. 13. Mice administered with 35% alcohol exhibited severe liver inflammatory lesions, characterized by congestion of hepatic blood vessels (Fig. 13B). Mice administered to alcohol and supplemented with 200 mg/kg vitamin B_{12} showed evidence of mild congestion of hepatic blood vessels (Fig. 13C). On the other, mice administered with alcohol and treated with a combination of vitamin B_{12} and CoQ_{10} revealed normal hepatic cellular architecture (Fig. 13D–E).

Vitamin B_{12} and CoQ_{10} protected brain cells from alcohol-induced gliosis

Neuropathological changes in the brain are shown in Fig. 14. Brains from the control group of mice revealed normal brain histo-architecture (Fig. 14A). Oral administration of mice with alcohol, resulted in focal areas of gliosis (Fig. 14B). It was also observed that

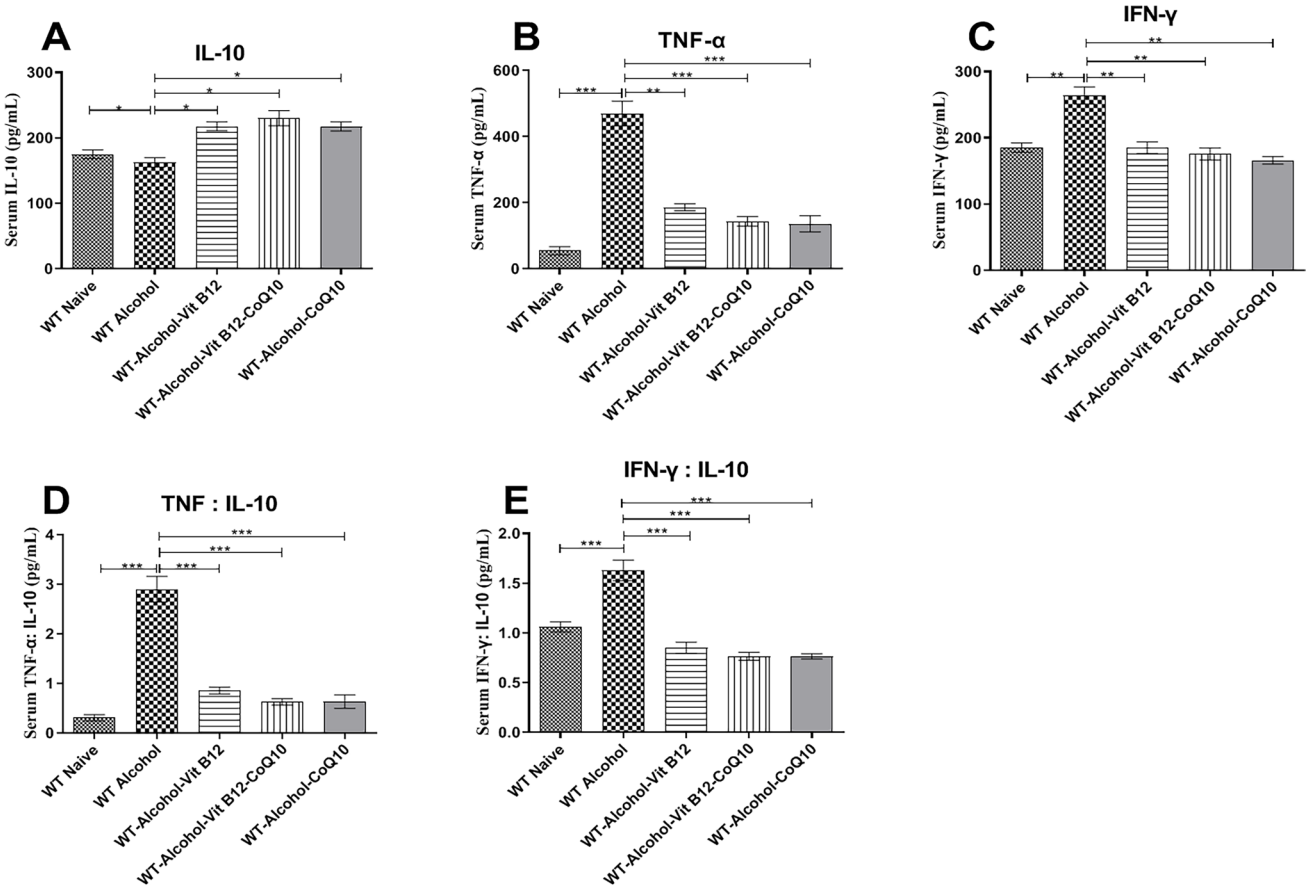

Fig. 7 Effects of vitamin B_{12} and CoQ_{10} on cytokine levels following alcohol-induced toxicity in Swiss albino mice. Serum cytokine levels of TNF-α (**A**), IFN-γ (**B**), and IL-10 (**C**). The TNF-α and IL-10 ratio (**D**) and INF-γ and IL-10 ratio (**E**). Comparisons between various groups were done using one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$; $**p \leq 0.001$ $***p \leq 0.0001$). Bars represent mean ± SEM

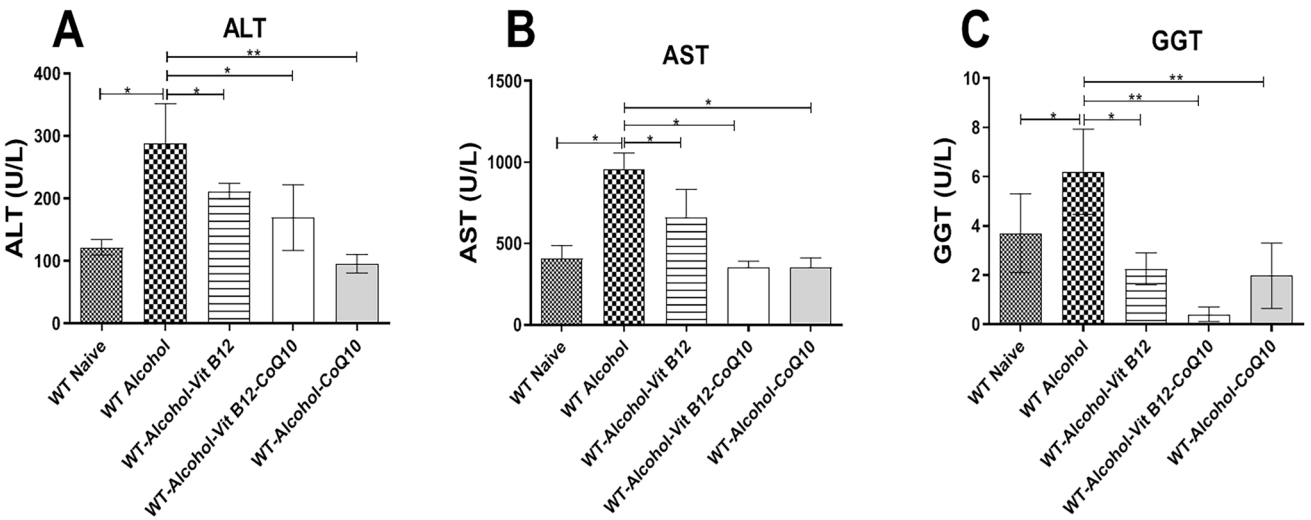

Fig. 8 Mean levels of ALT, AST, and GGT following administration of vitamin B_{12} and CoQ_{10} following alcohol-induced toxicity in Swiss albino mice. Serum levels of ALT (**A**), AST (**B**), and GGT (**C**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $*p \leq 0.05$; $**p \leq 0.001$). Bars represent mean ± SEM

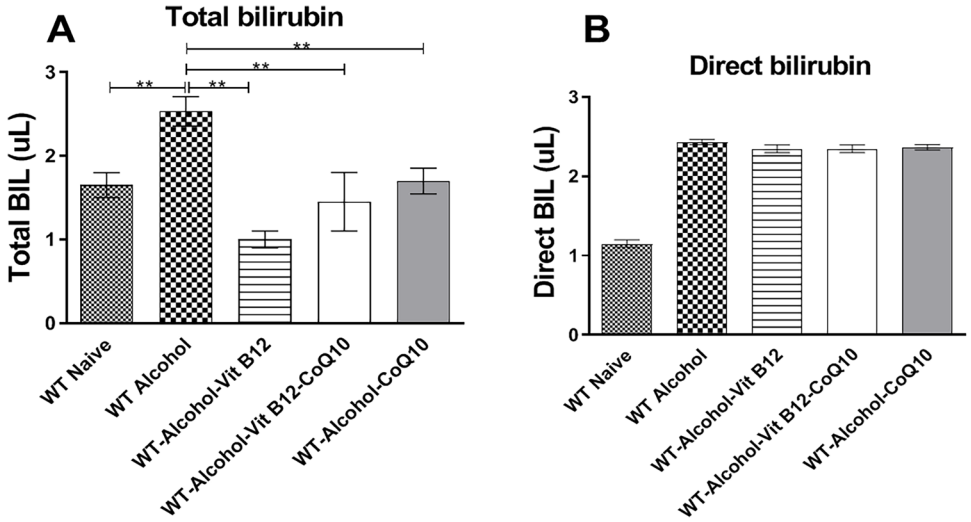

Fig. 9 Effects of supplementation of vitamin B_{12} and CoQ_{10} on bilirubin levels following alcohol-induced toxicity in Swiss albino mice. Serum levels of total bilirubin (**A**) and direct bilirubin (**B**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $^{**}p \leq 0.001$). Bars represent mean ± SEM

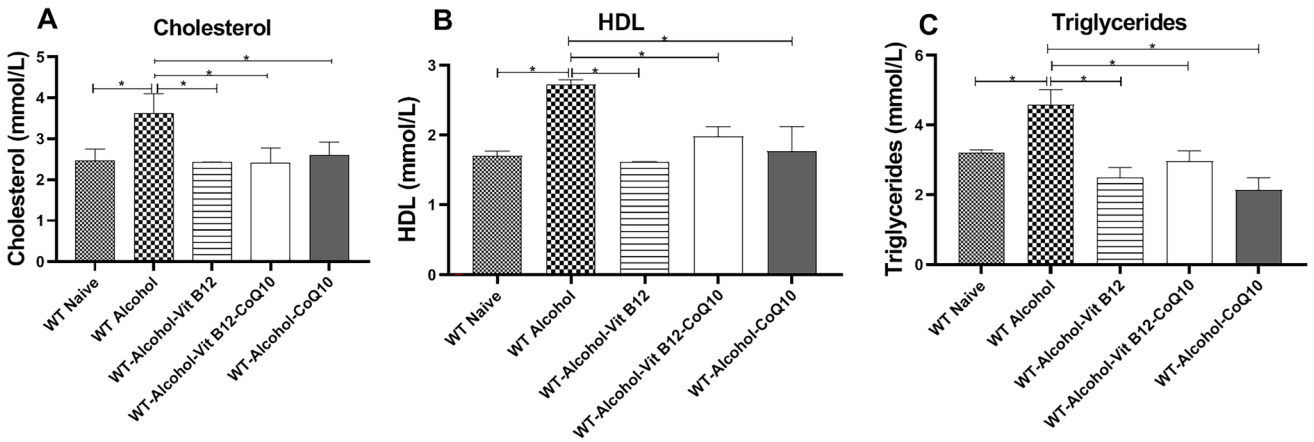

Fig. 10 Effects of supplementation of vitamin B_{12} and CoQ_{10} on lipid profile following alcohol-induced toxicity in Swiss albino mice. Serum levels of total cholesterol (**A**), HDL (**B**), and triglycerides (**C**). Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $^{*}p \leq 0.05$). Bars represent mean ± SEM

alcohol-administered mice that were treated with vitamin B_{12} and CoQ_{10} had normal brain architecture without any observable evidence of inflammation (Fig. 14C–E).

Discussion

Findings from this study clearly show that alcohol intake devastates vital physiological and biochemical processes critical in normal growth and development. Most importantly, the administration of vitamin B_{12} and CoQ_{10} provided a significant level of protection from alcohol-induced toxicities. In this study, we established that vitamin B_{12} and CoQ_{10} have the ability to assuage alcohol-induced hematological changes, alteration of lipids, oxidative stress, and inflammation in a mouse model.

Mice administered with alcohol registered a significant decrease in general body weight. Similarly, mice administered with vitamin B_{12} had reduced weight gain, which may be associated with the ability of vitamin B_{12} to lower plasma levels of lipids [26]. Mice that received a combination of vitamin B_{12} and CoQ_{10} did not show marked weight change. Alcohol-induced interference with weight has in the recent past been linked to poor appetite and food intake, interference with brown adipose tissue growth, and energy metabolism [27]. Based on previous studies, it was not surprising that the group treated with CoQ_{10} did not show significant weight gain. Past studies have shown CoQ_{10} inhibition of lipid accumulation and adipocyte differentiation, resulting in weight loss in mice supplemented with CoQ_{10} [28, 29].

Previous studies have noted an increase in liver weight in mice exposed to alcohol as well as hepatic enzyme

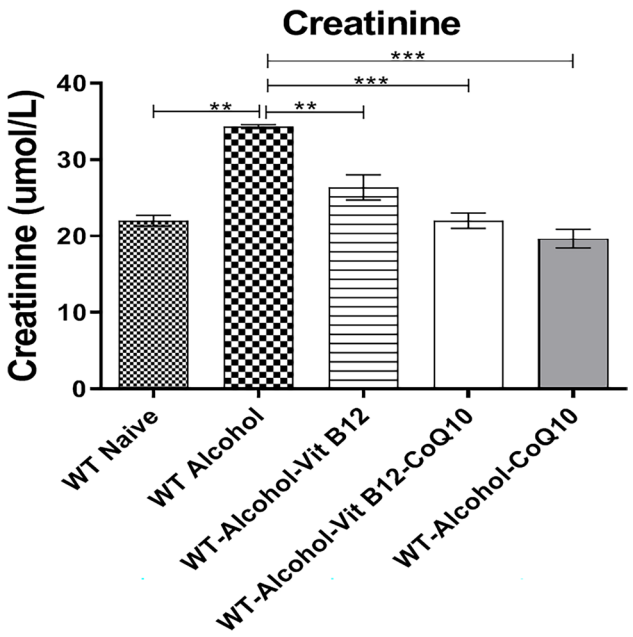

Fig. 11 Determination of the effect of alcohol, vitamin B_{12} and CoQ_{10} on serum creatinine levels. Comparisons between various groups were done by one-way ANOVA with Tukey multiple comparisons post hoc test (indicated level of significance: $^{**}p \leq 0.001$ $^{***}p \leq 0.0001$). Bars represent mean ± SEM

induction, and morphological changes in the hepatocytes [30]. In the current study, supplementation with vitamin B_{12} and CoQ_{10} abolished alcohol-induced liver due to their anti-inflammatory and antioxidant properties.

Other studies have shown that xenobiotic chemicals such as alcohol induce kidney weight increase due to inflammation of nephrons [31]. In the current study, mice administered with alcohol had a significant increase in the weight of the kidney and spleen relative to the group of mice co-administered with vitamin B_{12} and CoQ_{10}. The capacity of vitamin B_{12} and $CoQ_{10,}$ when co-administered to block an alcohol-driven increase in kidney weight and splenomegaly could be attributed to their ability to attenuate alcohol-induced renal injury through the amelioration of oxidative stress and inflammation.

An increase in spleen weight in mice administered with alcohol has been observed previously [32]. Indeed, previous studies on animals have demonstrated that CoQ_{10} has the ability to prevent splenomegaly during trypanosome infection [22]. Another previous study showed the ability of CoQ_{10} to protect mice against hepatomegaly and splenomegaly due to a Friend Leukemia viral infection [33]. These observations corroborate findings from the present study in which mice exposed to alcohol and supplemented

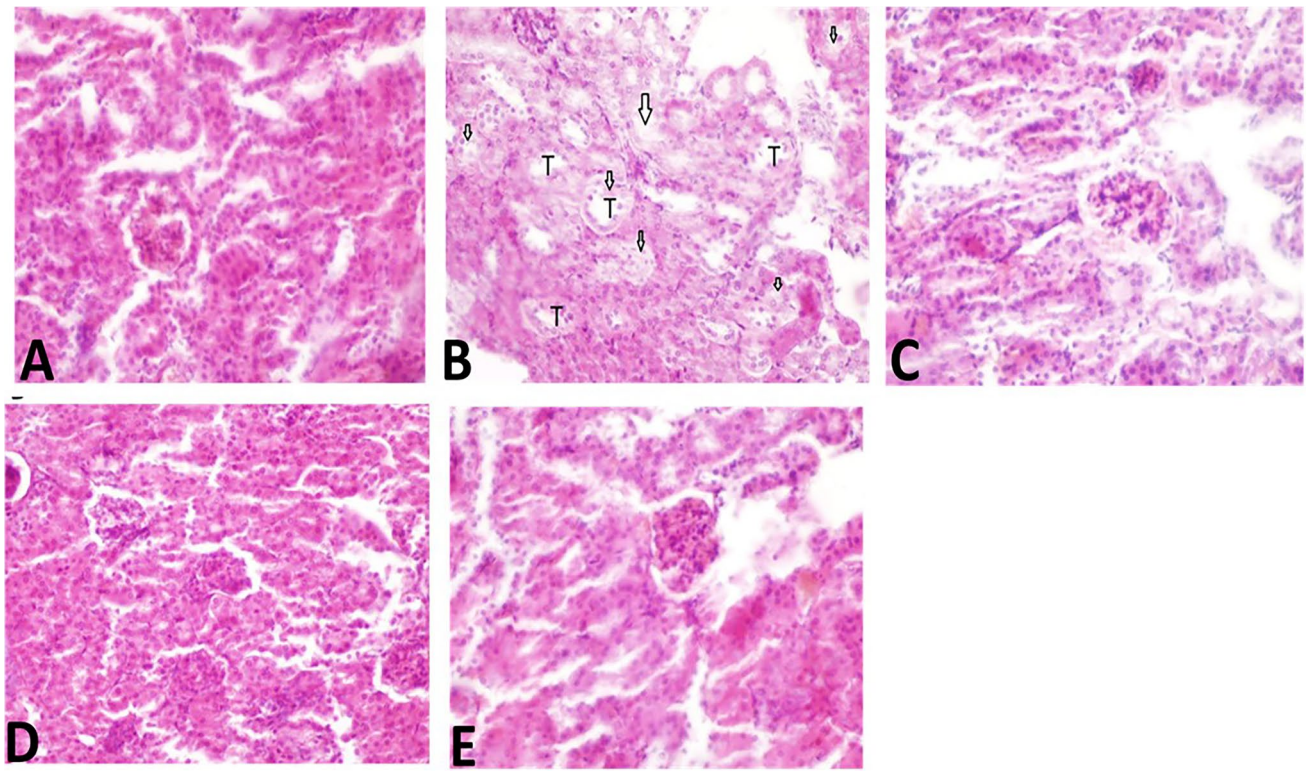

Fig. 12 The figure depicts histological sections of kidney tissue following oral administration of alcohol, vitamin B_{12}, and CoQ_{10}. Kidney organs from the control group (**A**), alcohol group (**B**), alcohol and vitamin B_{12} group (**C**), alcohol-vitamin B_{12} and CoQ10 (**D**), and alcohol and CoQ10 group (**E**) were harvested and processed for histological examination using H&E staining. Arrows show regions of focal and multifocal tubular epithelium and interstitial hemorrhage. H&E staining: original magnification × 400

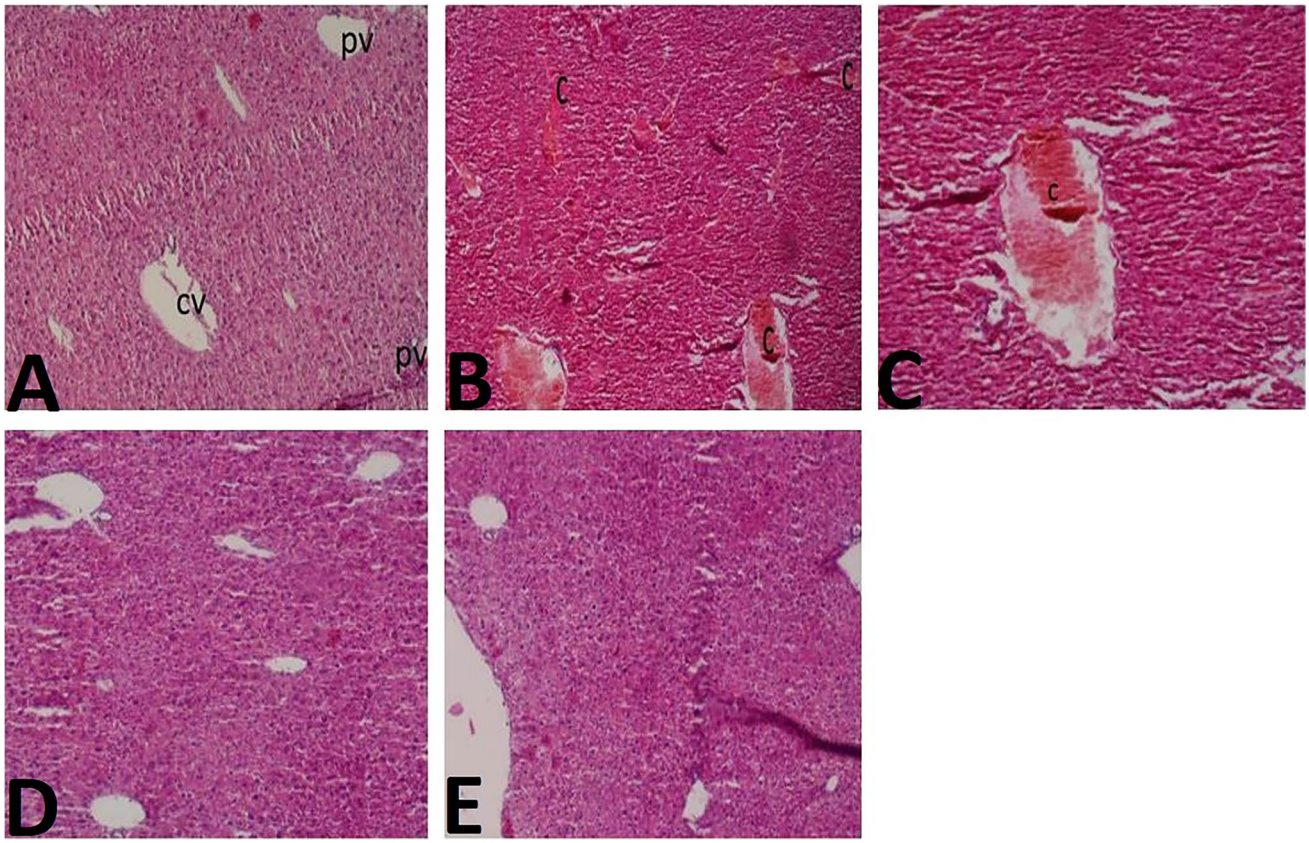

Fig. 13 The figure shows the effects of alcohol on the liver and the hepato-protective role of vitamin B_{12} and CoQ_{10}. Liver organs from the control group (**A**), alcohol group (**B**), alcohol and vitamin B_{12} group (**C**), alcohol-vitamin B_{12} and CoQ10 (**D**), and alcohol and CoQ10 group (**E**). The tissues were processed for histology with H&E staining. Images show representative liver sections (original magnification × 100) with the hepatocyte necrosis indicated by the arrows

with CoQ_{10} showed a marked decrease in hepatomegaly and splenomegaly. Based on our experimental design, such benefits by CoQ10 can be clearly attributable to its antioxidant and anti-inflammatory properties. The power of CoQ10 to preserve GSH in the presence of toxic chemicals, a potent antioxidant majorly synthesized in the liver, may be vital.

Hematopoietic processes are extremely vital in the production of important blood cells. It was therefore important to determine how alcohol affects vital blood cells and any potential beneficial effects of vitamin B_{12} and CoQ_{10}. Previously, it has been demonstrated that rats exposed to 20% ethanol had a significant reduction in RBC counts [34]. It is well-established that chronic consumption of alcohol is central to the pathogenesis of the alcoholic liver disease, which is associated with anemia [35].

Furthermore, chronic alcohol ingestion damages the stomach lining and intestines, thus impairing nutrient absorption and transport into the blood. Similarly, nutritional deficiencies alter folate, glucose, and sodium absorption [36]. The significant reduction in RBC is an indication of their possible destruction by reactive oxygen species produced during alcohol metabolism. Note that RBCs are polyunsaturated and lack nuclei and are devoid of reducing powers by NADPH. Therefore, RBCs are susceptible to lipid peroxidation and oxidative stress [37]. Clearly, a powerful antioxidant and anti-inflammatory compound may potentially benefit RBCs in the presence of alcohol.

From the current study, it is evident that alcohol administration suppressed RBC, hemoglobin (HB), and hematocrit. Notably, our data shows that there was a significant improvement in RBC, HGB, and hematocrit parameters after administration of vitamin B_{12} and CoQ_{10}, either alone or in combination following alcohol exposure. This is not surprising given that vitamin B12 has reactive oxygen species scavenging activity and modulates cytokines, which can induce inflammation. Moreover, it reduces homocysteine-induced ROS, ultimately protecting blood from glycation end products [17]. Previous studies have shown that CoQ_{10} protects RBCs from oxidative stress-induced hemolysis [22, 38, 39]. The present data are in tandem with other studies that have shown that CoQ_{10} supplementation restores RBCs,

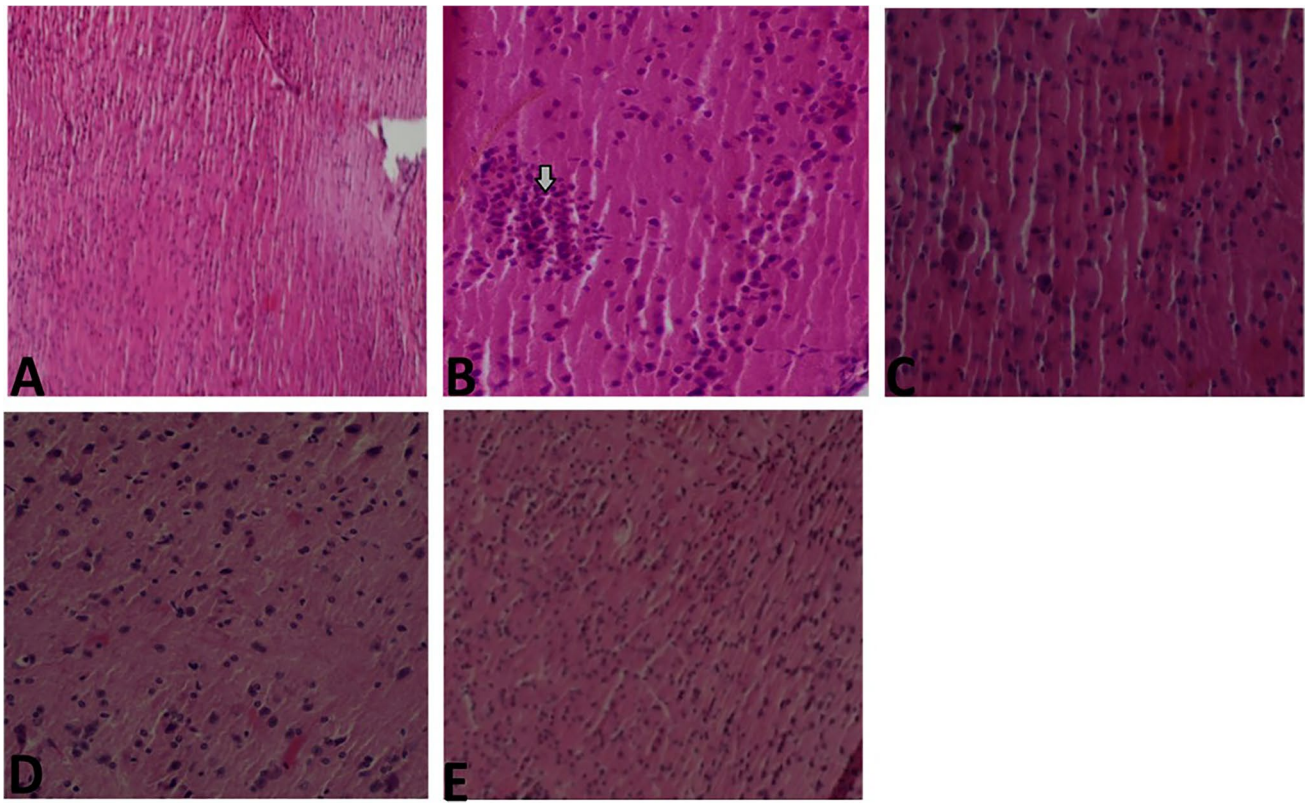

Fig. 14 The figure shows the effect of vitamin B_{12} and CoQ_{10} on mice brain tissues following alcohol-induced toxicity. Brain tissues from the control group (**A**), alcohol group (**B**), alcohol and vitamin B_{12} group (**C**), alcohol-vitamin B_{12} and CoQ10 (**D**), and alcohol and CoQ10 group (**E**). The tissues were processed for histology with H&E staining. Images show representative brain sections (original magnification × 100). Arrows show regions of facial gliosis

HGB, and hematocrit levels [22]. Additionally, a study by Molina et al. [40] showed that vitamin B_{12} plays a key role in erythropoiesis. These findings indicate the significant role of vitamin B_{12} and CoQ_{10} either alone or in combination in reversing the adverse effects of alcohol-induced alteration of the hematopoietic process.

Additional findings from this study show that the total WBC were significantly elevated in mice administered with alcohol when compared with other treatment groups. However, WBC phenotypes like lymphocytes, neutrophils, monocytes, eosinophils, and basophils were comparable across all treatment groups. The results of the present study are consistent with a previous study that noted a significant alcohol-induced elevation of total WBC without affecting the number of eosinophil and lymphocytes [41]. Remarkably, supplementation with either vitamin B_{12} or CoQ_{10} alone or in combination restored the normal levels of the total WBC. These results may suggest that vitamin B_{12} and CoQ_{10} administration triggers a marked balance in the immunostimulation of the lymphoproliferative responses. However, this merits further investigation.

Oxidative stress is intimately implicated in the pathological progression of alcohol-induced liver disease [42]. Alcohol metabolism results in active oxidative stress due to its ability to accelerate the rise in reactive oxygen species (ROS) during ethanol conversion to acetate. Previous studies have shown that ethanol metabolism is directly involved in the production of reactive oxygen species and reactive nitrogen species (RONS) with concomitant depletion of GSH and a decrease in antioxidant activity [43]. Moreover, it has been shown that alcohol intake triggers congestion of red pulp and decreases white pulp resulting in splenomegaly [44].

Reactive oxygen species produced during spontaneous metabolism are scavenged and quenched by antioxidant defense systems such as reduced glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase. Additionally, this defense system also participates in the termination of the chain reactions from free radicals [13]. Accumulation of ROS is known to induce oxidative damage to hepatocytes and thus the primary mechanism of alcohol-induced liver injury [45]. Herein, the alcohol-driven development of oxidative stress was abrogated by oral administration of vitamin B_{12} and CoQ_{10}. Specifically, vitamin B_{12} and CoQ_{10} demonstrated a significant capacity to stabilize the concentration of liver, kidney, spleen, brain, and lung GSH levels in the presence of alcohol. Note that GSH levels were

depleted by alcohol administration. Indeed, previous studies have shown that chronic GSH depletion is a key event in alcohol liver steatosis [46]. Similarly, a study by Kołota et al. [47] showed that mice administered with alcohol had significantly decreased levels of GSH relative to the normal control.

Previous studies have shown that vitamin B_{12} administration restored GSH levels following acetaminophen-induced toxicity [48]. According to Marques et al. [49], CoQ_{10} administration has been shown to protect from toxicity-induced GSH depletion. It is therefore plausible that the administration of vitamin B_{12} and CoQ_{10} could protect the liver from ROS, produced by acetaldehyde arising from the metabolism of alcohol.

The oxidation of biomolecules usually occurs due to elevated levels of ROS [50]. In the present study, alcohol administration significantly depleted GSH levels in the kidney tissue. Supplementation of mice with vitamin B_{12} and CoQ_{10} separately or in combination, significantly restored GSH levels in the kidney. In the absence of vitamin B_{12} and CoQ_{10}, the alcohol-induced depletion of GSH would result in high levels of ROS and subsequently result in kidney damage [50]. Notably, the kidney consists of polyunsaturated fatty acids making it susceptible to ROS [16].

In the current study, GSH levels were used as an indicator for the presence and absence of oxidative stress. Findings demonstrate that alcohol resulted in the depletion of GSH in the brain, liver, lungs, and kidney. Most importantly, the depletion of GSH in these organs was reversed by vitamin B_{12} and CoQ_{10} when administered together or separately. It is vital to note that CoQ10 was superior to vitamin B_{12} in restoring GSH levels in these organs. Previous studies on humans have clearly shown that alcohol drives the depletion of GSH, reinforcing the fact that oxidative stress is a major event in alcohol-induced spleen and lung damage [51]. It is evident that with further studies, supplementation with vitamin B_{12} or CoQ_{10} may find medical application to ameliorate alcohol-induced organ damage.

Tumor necrosis factor-α (TNF-α) and interferon-gamma (INFγ) are pro-inflammatory cytokines that have previously been implicated in the progression of alcohol-induced hepatotoxic [52]. High levels of TNF-α produced by classically activated macrophages, during chronic alcohol consumption, facilitate the infiltration of lymphocytes in the liver accelerating liver injury [53]. In this study, both TNF-α and INF-γ were significantly upregulated in mice administered with alcohol. These findings corroborate previous studies in which alcohol was found to modulate cytokine production [6, 54], enhancing the release of IFN-γ and infiltration of Ly6G + into the cells, with consequent tissue injury [52, 55]. Supplementation of mice with vitamin B12 alone or in combination with CoQ_{10} significantly nullified alcohol-induced elevation of TNF-α and interferon INF-γ. A notable finding in the current study is the ability of vitamin B_{12} and CoQ_{10} to significantly modulate TNF-α, IFN-γ, and IL-10, thereby providing protection against alcohol-induced inflammatory processes. Mice orally administered with vitamin B_{12}/CoQ_{10} showed a balance between the pro-inflammatory and anti-inflammatory cytokines. Previous studies suggest that CoQ_{10} plays a key role in decreasing pro-inflammatory cytokines by inhibiting NF-κB gene expression and macrophage inflammatory protein [56]. Furthermore, CoQ_{10} promotes the production of adiponectin which inhibits the production of pro-inflammatory cytokines such as TNF-α. Additionally, vitamin B_{12} has been demonstrated to lower pro-inflammatory cytokine and increases the levels of anti-inflammatory cytokines [57]. Our data provide evidence that alcohol exposure promotes the elevation of inflammatory cytokines (TNF-α and INF-γ) with a concomitant decrease in anti-inflammatory cytokines (IL-10), and that supplementation with vitamin B_{12} and CoQ_{10} stabilizes cytokine levels in the presence of alcohol with vital implications in immune function and alcohol toxicity.

Liver damage due to alcohol exposure is a well-established phenomenon. In the present study, the ability of vitamin B_{12} and CoQ_{10} to protect from alcohol-induced liver injury in mice was investigated. Alcohol-induced liver injury was evident with a significant increase in AST, ALT, GGT, and bilirubin levels. Treatment with vitamin B_{12} and CoQ_{10} alone or in combination abrogated alcohol-driven elevation of AST, ALT, GGT, and bilirubin. Previous studies have shown vitamin B_{12} and CoQ_{10} to be hepato-protective against dimethylnitrosamine and N-nitrosodimethylamine-induced liver injury and hepatic fibrosis as well as lipopolysaccharide-induced toxicity [58–60]. Hepatic injury is usually characterized by the leakage of cellular enzymes into the plasma [61]. Our findings suggest that vitamin B_{12} and CoQ_{10} may aid liver recovery in the presence of alcohol perhaps due to their potent antioxidant and anti-inflammatory effects.

The kidney is involved in alcohol metabolism to a lesser degree when compared to the liver. Elevation of creatinine in serum is characteristically used as an indicator of kidney damage. In the current study, mice exposed to alcohol showed a significant elevation of creatinine, a finding consistent with other previous studies [62, 63]. It was interesting to note that vitamin B_{12} and CoQ_{10} attenuated alcohol-induced kidney damage, as demonstrated by near-normal creatinine levels.

This is an important finding given that previous studies have demonstrated that chronic alcohol consumption changes the kidney's glomerular morphology, affecting the glomerular filtration rate and consequently creatinine clearance rate [64]. Ethanol is associated with a reduction in kidney function by inducing multifocal vascular congestion [65]. Notably, previous studies have reported that CoQ_{10} has renal protective

effects [66], whereas vitamin B_{12} has been shown to ameliorate gentamicin-induced nephrotoxicity [16]. The ameliorative effects of vitamin B_{12} and CoQ_{10} may be attributed to their antioxidant and anti-inflammatory properties. In addition, histopathological analysis revealed that, unlike the control group, the group administered with alcohol manifested focal areas of tubular epithelium degeneration and congestion of renal blood vessels. Both single and combined administration of vitamin B_{12} and CoQ_{10} attenuated these effects on the renal tissue, thereby improving tubular and glomerular structures.

Chronic liver injury following alcohol toxicity was further confirmed by liver histopathological changes. It is evident from previous studies that chronic alcohol administration in mice causes lethal liver structural changes [13]. From our findings, it is evident that GSH depletion, the elevation of liver enzymes, and histopathological changes are vital hallmarks of alcohol-driven damage to the kidney. In the current study, hepatic blood vessel congestion was evident on the histological slides analyzed. The toxic effects on the liver cells were prevented by vitamin B_{12} and CoQ_{10}. In a previous study, the presence of vitamin B_{12} has been shown to inhibit inflammation and fibrosis [48]. The present data is consistent with previous studies reporting that supplementation of CoQ_{10} significantly ameliorated liver hepatotoxicity [67], while another similar study has shown that vitamin B_{12} can protect from acetaminophen-induced liver toxicity [48].

The histopathological observation of the brain revealed focal gliosis in mice administered with ethanol (Fig. 14). Such neuropathological features have been observed previously by Hamid et al. [68], where administration of alcohol (1.8 g/kg for 14 days) induced neurodegeneration in the brain cerebral cortex. Most importantly, mice that were supplemented with vitamin B_{12} and CoQ_{10} alone or in combination showed normal brain structural architecture. The alcohol-induced toxicity in the brain has been attributed to the presence of high levels of polyunsaturated fatty acids and iron in the brain [68]. Furthermore, the brain has high lipid content and aerobic activities in brain cells, making it vulnerable to neurotoxins and oxidative damage [69]. Previous studies have shown that vitamin B_{12} can scavenge reactive oxygen species, preserve glutathione, and reduce homocysteine-induced oxidative stress [17, 70].

Mice administered with alcohol had significantly higher total serum cholesterol, HDL, and triglyceride levels, consistent with previous studies [71]. However, treatment with vitamin B_{12} and CoQ_{10} following alcohol administration, either alone or in combination, stabilized cholesterol levels, HDL, and triglyceride levels. These results provide new evidence that vitamin B_{12} and CoQ_{10} supplementation could aid in the stabilization of alcohol-driven derangement of lipid metabolism. Further studies are needed to determine specific molecular processes that aid the recovery of lipid metabolism during alcohol intoxication driven by vitamin B_{12} and CoQ_{10}.

Conclusion

Chronic exposure to alcohol impairs vital physiological and biochemical processes vital for maintaining normal homeostasis in various organ systems, such as hematopoiesis, immune balance, and antioxidant potential. Notably, findings suggest promising possibilities for further investigations and development to deploy vitamin B12 and CoQ10 as adjunct therapy alongside existing pharmacological interventions for treating and preventing alcohol toxicities in humans.

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Author contribution BK, VKM, and JNN performed experiments; BK, GO, and JNN analyzed and interpreted the experimental results; AOI provided the laboratory facility and helped in the designing of experimental procedures BK wrote the manuscript. MG, MG, AOI, and JNN edited the manuscript. All authors read and approved the final manuscript.

Data availability All data generated and analyzed from this study are included in this manuscript or are available through the corresponding author upon request.

Declarations

Competing interests The authors declare no competing interests.

Ethics approval All experimental procedures and protocols involving mice were reviewed by the Institutional Review for approval Committee (IRC) of the Institute of Primate Research Karen, Kenya (ISERC/08/2017). All experiments were conducted in compliance with the recommendations of the Helsinki Declaration on guiding principles on the care and use of animals.

Conflict of interest The authors declare no competing interests.

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