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Original Article *Toxicology*

Alpha-tocopherol and quercetin modulate primary hemodynamic parameters, oxidative stress indices, and biomarkers of cardio-renal functioning in ethanol-exposed rats

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ABSTRACT

Objectives: The objective of this study was the assessment of the ameliorative roles of alpha-tocopherol and quercetin on the toxic mechanisms associated with ethanol (EtoH) exposure in the cardiac and renal systems of rats.

Material and Methods: Forty male rats were randomly selected and divided into five groups as follows: 0.2 mL distilled water; EtoH 40% v/v; EtoH + 2.5 mg/kg alpha-tocopherol; EtoH + 50 mg/kg quercetin; and EtoH + a cocktail of alpha-tocopherol and quercetin.

Results: Treatment with alpha-tocopherol and quercetin significantly ($P < 0.05$) ameliorated EtoH-induced alterations in hemodynamic and electrocardiographic parameters, kidney function markers, and antioxidant defense status of rats with significantly elevated levels of glutathione, glutathione peroxidase, superoxide dismutase, and glutathione-S-transferase observed in antioxidant-treated rats. Histopathologic lesions induced by EtoH including focal loss of myofiber striation, degeneration, and infiltration of inflammatory cells in the cardiac tissues, as well as patchy tubular necrosis, congestion, and ectasia in renal tissues were absent in the antioxidant treated rats. Heightened immunohistochemical expressions of cardiac-specific troponin and angiotensin converting enzymes induced by EtoH were abated by alpha-tocopherol and quercetin treatment.

Conclusion: Alpha-tocopherol and quercetin mitigated oxidative stress-mediated ethanol-induced derangements of the cardiovascular and renal systems in rats.

Keywords: Alpha-tocopherol, Quercetin, Ethanol, Necrosis, Troponin

INTRODUCTION

The exposure to ethanol (EtoH) has continued to pose significant global public health concerns, with excessive EtoH intake unequivocally associated with an increased risk of cardiovascular disease.[1,2] Overwhelming increases have been recorded in the rates of alcohol consumption,

with skyrocketing implications on health and general well-being, and positive correlation reported between increased alcohol consumption, all-cause mortality, and cancers.[3,4] Unfortunately, moderate to high exposure induces complex changes in blood biochemistry and alterations in many biomarkers for cardiometabolic risk.[5,6] Furthermore, alcohol exposure has been positively correlated with incidences of cardiomyopathy, high blood pressure, increased risk of myocardial infarction, arrhythmias, fatal cardiac arrest, and stroke.[7]

Epidemiologic, clinical, and experimental reports abound in literature to underline the compelling evidence for alcohol-induced hypertension.[8] Moreover, alcohol-induced heightened blood pressure parameters have been linked with the potentiation of the renin-angiotensin aldosterone system with consequent increases in peripheral resistance, expansion of blood volume, and induction of oxidative stress in vascular endothelium.[9,10] In the same vein, irreversible toxicological changes and loss of renal tubular functioning have been associated with excessive EtoH exposure,[11] through the potentiation of oxidative processes on proteins and deoxyribonucleic acid in the kidneys.[12,13]

Antioxidants such as chrysin, phenolic acids, and flavonoids, as well as antioxidant-rich medicinal plants such as *Panax ginseng* and *Silybum maximum* have been reported to significantly mitigate oxidative-stress-mediated tissue damage associated with alcohol exposure.[13,14] Alpha-tocopherol and quercetin are potent dietary antioxidants.[14,15] Alpha-tocopherol, a fat-soluble non-enzymatic antioxidant, inhibits lipid peroxidation reaction and prevents oxidative damage in cell membranes of various mammalian tissues.[16-21] Quercetin occurs naturally in many fruits and vegetables and has been reported to scavenge free radicals and mitigate pathogenic oxidative mechanisms *in vivo*.[22] The objective of this study was the assessment of the ameliorative roles of alpha-tocopherol and quercetin on the toxic mechanisms associated with EtoH exposure in the cardiac and renal systems of rats.

MATERIAL AND METHODS

Experimental animals

Following an acclimatization period of 2 weeks, Groups 1–5 of eight animals each were assigned as follows: Control; EtoH 40% v/v, 5 g/kg;[23] EtoH and alpha-tocopherol 2.5 mg/kg;[24] EtoH and quercetin 50 mg/kg;[25] and EtoH and a cocktail of alpha-tocopherol/quercetin. Alcohol and quercetin were dissolved in distilled water, whereas alpha-tocopherol was dissolved in corn oil. The administration period lasted for 40 consecutive days.

Ethical approval

The experiment was carried out in accordance with ethical standards laid down by the University of Ibadan Animal Care Use and Research Ethics Committee with the approval number UI-ACUREC/TOA/20/0036.

Primary hemodynamic parameters

The primary hemodynamic parameters are as follows: Mean, diastolic and systolic blood pressures, and heart rate were monitored in non-anesthetized rats with a plethysmograph (CODA, Kent scientific), whereas indices of cardiac performance including the P-wave and QRS duration, QT interval, and R wave amplitude were obtained with an electrocardiograph.[26]

Preparation of serum and tissues for biochemical assays

The heart and kidney samples were harvested and kept at −4°C immediately till the time of analysis. The serum was also collected after the blood samples were clot and stored immediately at −4°C.

Homogenization

Kidney and heart tissues, from freshly sacrificed rats were homogenized, in 0.1 M phosphate buffer, with a Teflon homogenizer, and the resulting homogenate was centrifuged in a cold centrifuge at 10,000 rpm for 10 min to obtain post-mitochondrial fraction used for analysis of the markers of oxidative stress and systemic antioxidants.

Analysis oxidative stress markers and systemic antioxidants

The assay for oxidative damage in cardiac and renal tissues was determined as previously described.[27] The thiol contents and reduced glutathione (GSH) level were assayed as previously described,[28] whereas hydrogen peroxide generation and superoxide dismutase (SOD) level was measured as described by Wolff.[29] and Misra and Fridovich,[30] respectively. Glutathione S-transferase (GST), glutathione peroxidase (GPx), nitric oxide (NO), and myeloperoxidase (MPO) were assayed in accordance with the methods Habig *et al*.,[31] Beutler *et al*.,[32] Miranda *et al*.,[33] and Xia and Zweierm,[34] respectively. The blood urea nitrogen (BUN) and creatinine were determined using standard methods as highlighted in the manufacturer's instructions on their respective kits (Randox Ltd, UK).

Preparation of tissues for histopathological and immunohistochemical evaluation

Small pieces of kidney and heart were fixed in 10% formalin, embedded in paraffin wax, and sections of 5–6 mm in thickness

were made and, thereafter, stained with hematoxylin and eosin for histopathological examination according to the methods as previously described.[35] The tissues were subsequently used to evaluate the immunohistochemical expressions of cardiac specific troponin I (cTn I) in renal tissues and angiotensin-converting enzyme (ACE) in renal tissues of rats.

Statistical analysis

The data obtained from the experimental animals were analyzed using descriptive statistics. Analysis of variance with Dunnett's post-test was used to analyze statistical significance difference in the groups. A statistical package (Prism 8.0) was used to analyze the data.

RESULTS

The result of the effects of the administered antioxidants (alpha-tocopherol and quercetin) on alcohol-induced alterations of arterial pressures and electrocardiographic changes is presented in Tables 1 and 2, respectively. Alcohol altered the hemodynamic parameters, that is, the arterial pressures and heart rate, in comparison with control, whereas alpha-tocopherol and quercetin significantly ameliorated the alcohol-induced alterations, with near-normal values recorded with the coadministration of alpha-tocopherol and quercetin. Furthermore, alcohol induced a significant decrease in PR and QT intervals, and the duration of QRS complex relative to alpha-tocopherol and quercetin treated rats.

The result of the effects of the administered antioxidants (alpha-tocopherol and quercetin) on alcohol-induced alterations of markers of oxidative stress in cardiac and renal tissues of rats is presented in Figures 1-4. Alcohol induced a significant elevation of hydrogen peroxide, malondialdehyde (MDA), and sulfhydryl thiol content, but the opposite effect was seen with the analyzed systemic antioxidants reduced GSH, GPx, SOD, and GST [Figures 5-8]. However, alpha-tocopherol and quercetin significantly ameliorated these effects, with near to normal levels of oxidative stress markers recorded in rats administered both alpha-tocopherol and quercetin. In addition, the levels of serum MPO, urea, and creatinine were significantly increased by antioxidant treatment [Figures 9-11].

Histopathologic lesions induced by EtoH include focal loss of myofiber striation, degeneration, and infiltration of inflammatory

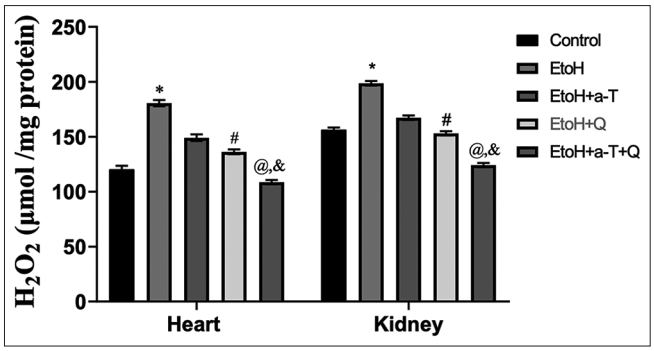

Figure 1: Hydrogen peroxide level in cardiac and renal tissues. Superscript (*) indicates significant ($P < 0.05$) increase compared with control; # indicates significant ($P < 0.05$) decrease compared with ethanol (EtoH); @ indicates significant ($P < 0.05$) decrease compared with EtoH+α-T; '&' indicates significant ($P < 0.05$) decrease compared with EtoH+Q.

Table 1: Ethanol-induced alterations of primary hemodynamic parameters and effects of antioxidants.

Parameter	Control	EtoH	EtoH+α-T	EtoH+Q	EtoH+α-T+Q
Systolic BP	121.5±3.2	186.6±1.3[a]	141.6±2.1[b]	149.6±4.8[b]	115.5±3.4[c]
Diastolic BP	93.5±2.6	148.0±5.6[a]	95.6±2.7[b]	104.8±5.9[b]	81.18±1.7[c]
Mean BP	104.0±2.36	160.8±3.8[a]	110.8±1.7[b]	118.2±5.3[b]	92.4±2.0[c]
Heart rate	352.5±10.5	375.2±11.9[a]	392.8±10.7[b]	364.3±9.1[b]	381.8±12.2[c]

Blood pressure (BP) measured in millimeter of mercury (mmHg), Heart rate measured in beats per minute. EtoH=Ethanol (40%); α-T=alpha-tocopherol (2.5 mg/kg); Q=quercetin (50 mg/kg). [a]significant ($p<0.05$) difference compared with control, [b]significant ($p<0.05$) difference compared with ethanol (EtOH), [c]significant ($p<0.05$) difference compared with EtoH+Q.

Table 2: Alcohol-induced alterations of electrocardiographic parameters and effects of antioxidants.

Parameter	Control	EtoH	EtoH+α-T	EtoH+Q	EtoH+α-T+Q
P wave (ms)	20.1±0.3	21.3±1.4	20.4±1.2	21.2±0.7	19.8±1.2
PR interval (ms)	33.4±1.2	29.8±1.2[a]	31.4±1.2	30.4±1.2	32.8±1.6
QT interval (ms)	96.0±6.1	62.4±3.2[a]	80.5±7.2[b]	76.4±3.8[b]	92.1±8.3[b]
QRS duration (ms)	18.2±1.3	12.7±4.6[a]	14.3±2.1[b]	15.3±1.8	17.5±2.1[b]
QTC (ms)	121.6±3.4	138.4±6.2[a]	92.1±3.7	111.4±2.5	108.4±2.9

EtoH=Ethanol (40%); α-T=alpha-tocopherol (2.5 mg/kg); Q=quercetin (50 mg/kg). P wave = duration of atrial depolarization; PR interval = duration of atrial depolarization and delay of impulse conduction in the atrioventricular node; QT interval = duration of ventricular depolarization and repolarization; QRS duration = total time for the depolarization of all parts of the ventricle; QTC = corrected QT interval. [a]significant ($p<0.05$) difference compared with control. [b]significant ($p<0.05$) difference compared with ethanol (EtOH).

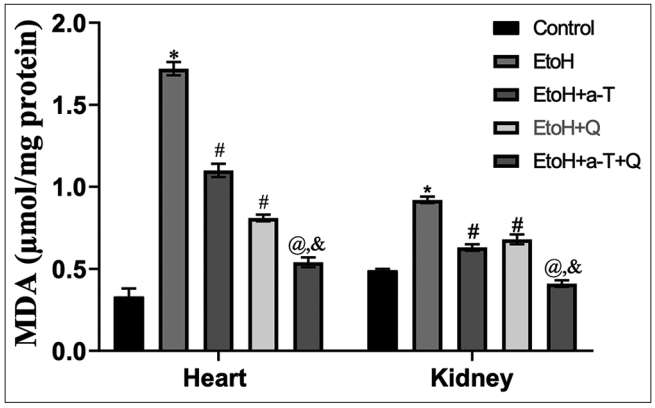

Figure 2: Malondialdehyde level in cardiac and renal tissues. Superscript (*) indicates significant ($P < 0.05$) increase compared with control; # indicates significant ($P < 0.05$) decrease compared with ethanol (EtoH); @ indicates significant ($P < 0.05$) decrease compared with EtoH + α-T; '&' indicates significant ($P < 0.05$) decrease compared with EtoH+Q. MDA: malondialdehyde.

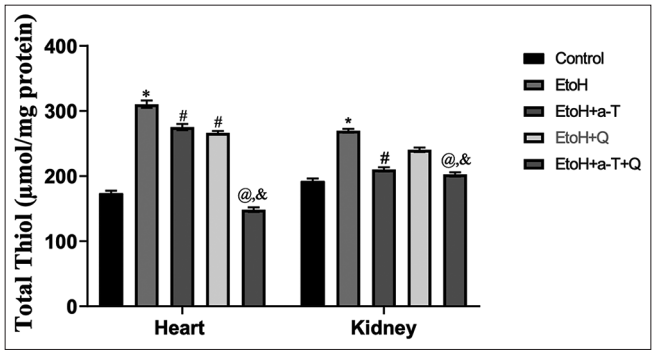

Figure 3: Total Tthiol level in cardiac and renal tissues. Superscript (*) indicates significant ($P < 0.05$) increase compared with control; # indicates significant ($P < 0.05$) decrease compared with ethanol (EtoH); @ indicates significant ($P < 0.05$) decrease compared with EtoH+α-T; '&' indicates significant ($P < 0.05$) decrease compared with EtoH+Q.

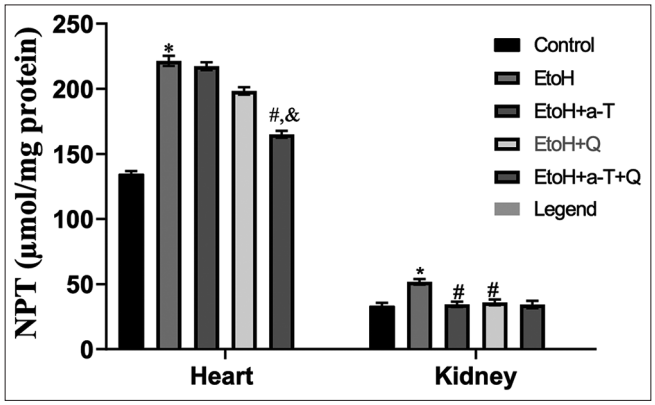

Figure 4: Non-protein thiol (NPT) in cardiac and renal tissues. Superscript (*) indicates significant ($P < 0.05$) increase compared with control; # indicates significant ($P < 0.05$) decrease compared with ethanol (EtoH); @ indicates significant ($P < 0.05$) decrease compared with EtOH+α-T; '&' indicates significant ($P < 0.05$) decrease compared with EtoH+Q.

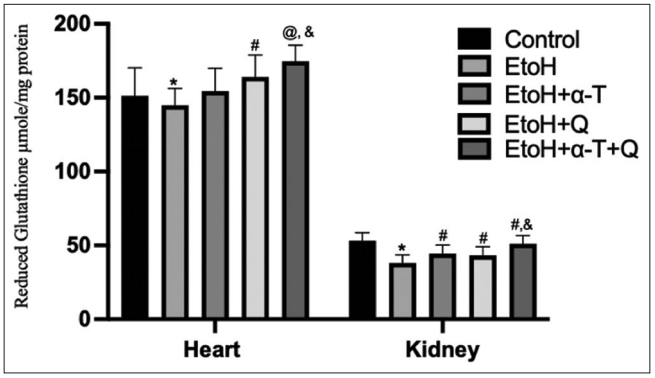

Figure 5: Reduced glutathione level in cardiac and renal tissues of rats. Superscript "*" indicates significant ($P < 0.05$) decrease compared with control; Superscript "#" indicates significant ($P < 0.05$) increase compared with ethanol (EtOH); Superscript "@" indicates significant ($P < 0.01$) increase compared with EtOH Superscript "&" indicates significant ($P < 0.05$) increase compared with EtoH+Q.

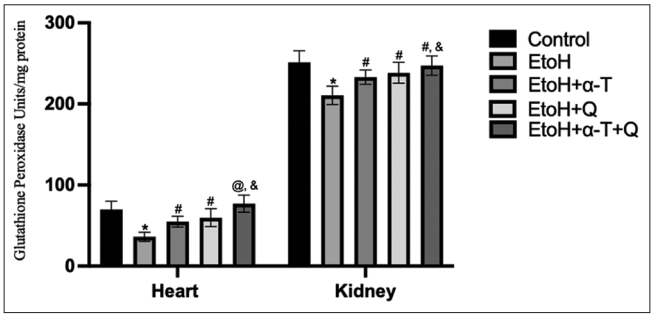

Figure 6: Glutathione peroxidase activity in cardiac and renal tissues of rats. Superscript "*" indicates significant ($P < 0.01$) decrease compared with control; Superscript "#" indicates significant ($P < 0.05$) increase compared with ethanol (EtOH); Superscript "@" indicates significant ($P < 0.01$) increase compared with EtOH Superscript "&" indicates significant ($P < 0.05$) increase compared with EtoH+Q.

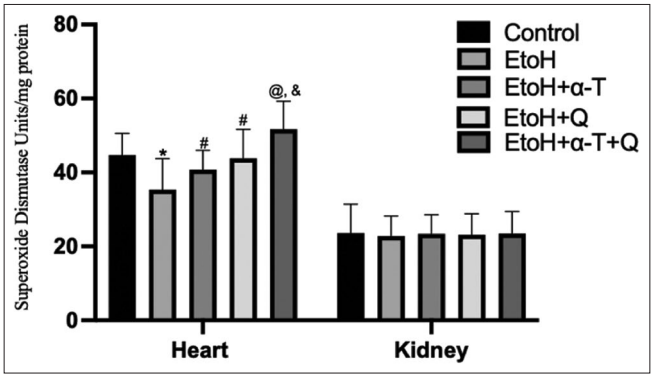

Figure 7: Superoxide dismutase activity in cardiac and renal tissues of rats. Superscript "*" indicates significant ($P < 0.01$) decrease compared with control; Superscript "#" indicates significant ($P < 0.05$) increase compared with ethanol (EtOH); Superscript "@" indicates significant ($P < 0.01$) increase compared with EtOH; Superscript "&" indicates significant ($P < 0.05$) increase compared with EtoH+Q.

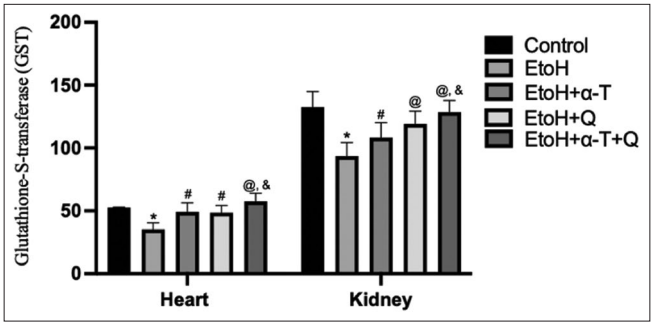

Figure 8: Glutathione (GSH)-S-transferase (mmole l-chloro-2, 4 dinitrobenzene-GSH complex formed/min/mg protein) activity in cardiac and renal tissues of rats. Superscript "*" indicates significant ($P < 0.01$) decrease compared with control; Superscript "#" indicates significant ($P < 0.05$) increase compared with ethanol (EtoH); Superscript "@" indicates significant ($P < 0.01$) increase compared with EtOH; Superscript "&" indicates significant ($P < 0.05$) increase compared with EtoH+Q.

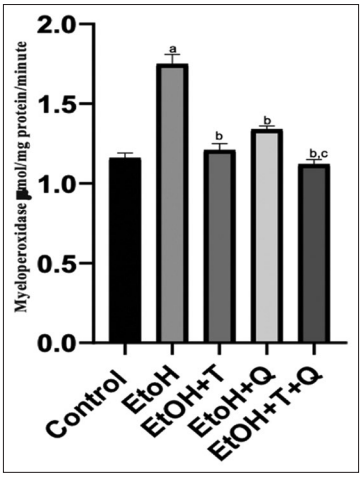

Figure 9: Alcohol-induced alteration of serum myeloperoxide and effect of antioxidants. Superscript a indicates significant ($P < 0.05$) increase compared with control; Superscript b indicates significant ($P < 0.05$) decrease compared with ethanol (EtoH); Superscript c; indicates significant decrease compared with EtOH+Q.

cells in the cardiac tissues, as well as patchy tubular necrosis, congestion, and ectasia in renal tissues. These were absent in the antioxidant treated rats [Figures 12 and 13]. Heightened immunohistochemical expressions of cTn [Figure 14] and ACEs [Figure 15] induced by EtoH were abated by alpha-tocopherol and quercetin treatment.

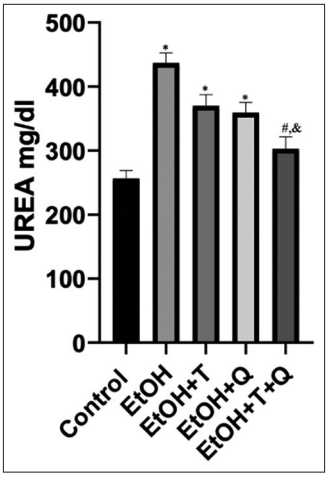

Figure 10: Alcohol-induced changes in urea concentration and effects of antioxidants. Superscript (*) indicates significant increase compared with control; Superscript (#) indicates significant decrease compared with ethanol (EtoH); Superscript (&) indicates significant decrease compared with EtoH+Q.

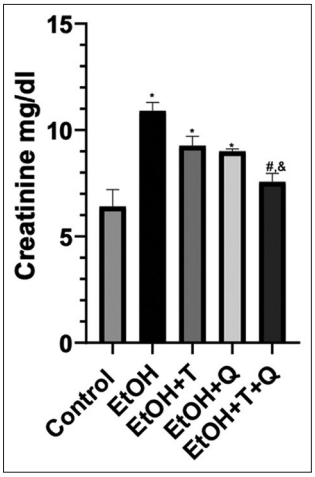

Figure 11: Alcohol-induced changes in creatinine concentration and effects of antioxidants. Superscript (*) indicates significant increase compared with control; Superscript (#) indicates significant decrease compared with ethanol (EtoH); Superscript (&) indicates significant decrease compared with EtoH+Q.

DISCUSSION

The oxidation of lipids in the *milieu intérieur* is modifiable by systemic antioxidative compounds that occur naturally in combinations with overall effects that are usually greater than that of individual agents acting alone and are

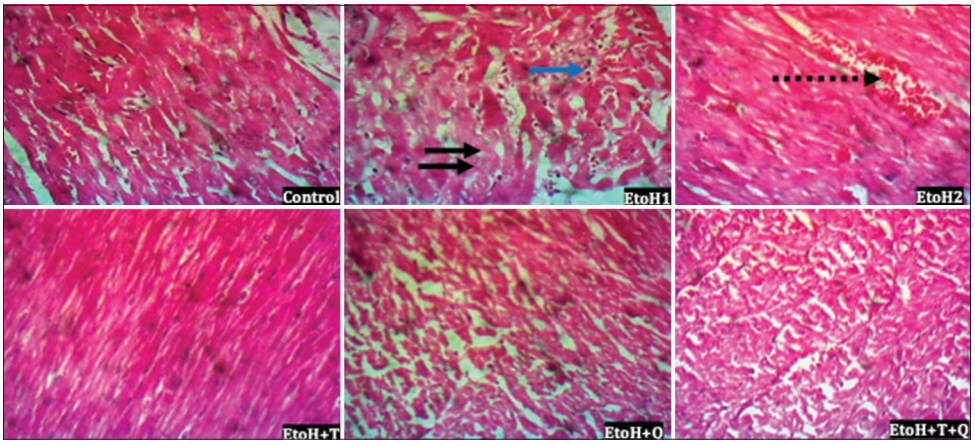

Figure 12: Photomicrograph of cardiac tissue showing focal loss of myofiber striation, degeneration (black arrows), a few infiltrate of inflammatory cells (blue arrow) and congestion (broken arrow); hematoxylin and eosin; Magnification ×400.

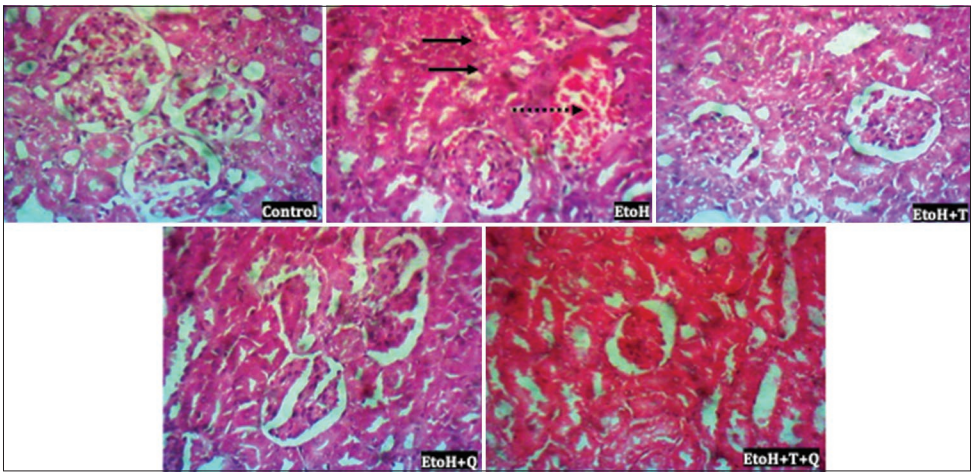

Figure 13: Photomicrograph of renal tissue showing patchy tubular necrosis and ectasia (thick black arrow) and congestion (broken arrow). Hematoxylin and eosin (H&E); Magnification ×400 Heart; H&E; Magnification ×400.

thus described as synergistic effects.[36] In this study, the modulatory and synergistic roles of two dietary antioxidants, alpha-tocopherol and quercetin, were investigated in EtoH-associated derangements of cardiovascular system. Redox imbalances have been prominently implicated in several multi organ-system pathologies including those of the cardiovascular and renal systems,[37] and alcohol has been reported to mediate some of its toxicities by tilting the redox balance in favor of the pro-oxidants particularly in the cardiorenal tissues. EtoH-induced oxidative stress may exacerbate the participation of reactive oxygen species (ROS) as second messengers in vascular endothelium-dependent function and remodeling of blood vessels; with the dysregulation of these physiological processes having considerable potential of contributing significantly to the development of vascular diseases.[38] When more than the coping capacity of the systemic antioxidants, the pro-oxidative agents cause significant deleterious effects on mammalian organs and tissues.[39] Therefore, the observation of attenuation of oxidative stress manifested by reduced lipid peroxidation, lowered hydrogen peroxide production, as well as lowered MPO and sulfhydryl thiols by alpha-tocopherol and quercetin suggest a potent activity for the administered dietary antioxidants to reduce oxidative stress, as previously described by various authors.[40,41]

In biological systems, alpha-tocopherol potently attenuates peroxidative damage in organs, with an inverse correlation reported between the level of the antioxidant and physiological derangements in the cardiovascular system.[42] Likewise, quercetin reportedly mitigates drug-instigated cardiomyopathy by positively modulating enzymatic antioxidative agents in rats.[43] In this study, the levels of the analyzed systemic antioxidants GSH, GPx,

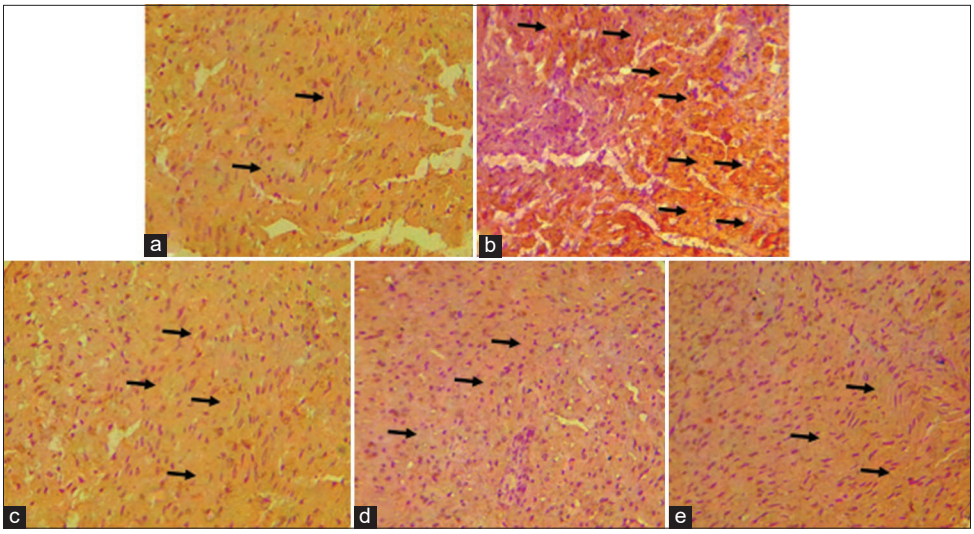

Figure 14: The immunohistochemistry of cardiac troponin. (a) Group A (Control), (b) Group B (Ethanol [EtoH] [40%]), (c) Group C (EtoH [40%] + alpha-tocopherol (2.5 mg/kg), (d) Group D (EtoH [40%] + quercetin 50 mg/kg), and (e) Group E (EtoH [40%] + alpha-tocopherol + quercetin 50 mg/kg). Black arrows indicate immunohistochemical expression of cardiac troponin. Slides stained with high definition hematoxylin. (Magnification ×100).

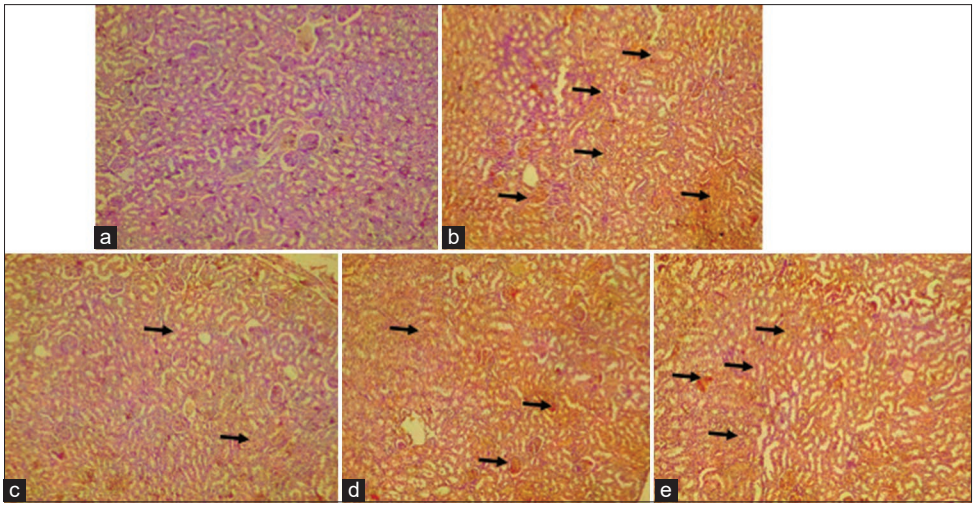

Figure 15: The immunohistochemistry of renal angiotensin converting enzyme. (a) Group A (Control), (b) Group B (Ethanol [EtoH] [40%]), (c) Group C (EtoH [40%] + alpha-tocopherol 2.5 mg/kg), (d) Group D (EtoH [40%] + quercetin 50 mg/kg), and (e) Group E (EtoH [40%] + alpha-tocopherol + quercetin 50 mg/kg). Black arrows indicate immunohistochemical expression of angiotensin converting enzyme. Slides stained with high definition hematoxylin. (Magnification ×100).

SOD, and GST are significantly reduced following alcohol exposure. Thus, it can be inferred that alcohol exerted a potent oxidative stress inducing activity by producing large amounts of ROS that required heightened mopping up by the systemic enzymatic and non-enzymatic antioxidants and consequent depletion of the agents for the restoration of physiological oxidant levels in cardiovascular and renal systems. Alcohol-induced oxidative stress is linked to the metabolism of EtoH involving both microsomal and mitochondrial systems, production of ROS and reactive nitrogen species, elevation of MDA, hydroxyethyl radical, and hydroxynonenal protein adducts, depletion of GSH levels, decreased antioxidant activity, modification of all biological structures, and serious malfunction of cells and tissues.[44] Moreover, hydrogen peroxide has been reported to increase the production of endogenous vasoconstrictors in blood vessels, thereby inducing increased arteriolar tone and consequently hypertension.[45] The potentiation of the antioxidant effect of alpha-tocopherol by quercetin and vice versa recorded in this study ratifies the submission of Punithavathi and

Prince,[46] who reported that the combination of the two antioxidants is synergistic and potently scavenge-free radicals, with the improvement of systemic antioxidant capacity and maintenance of calcium ion levels. High levels of alcohol in mammalian tissues downregulate endothelial NO synthesis, alter bioavailability of NO, and impair vascular relaxation in rats, thereby predisposing the cardiovascular system to hypertension and other diseases such as renal failure.[47,48] Therefore, the significantly elevated serum NO when rats were gavage with alpha-tocopherol and quercetin compared with rats exposed to the toxic effects of alcohol without antioxidant treatment further corroborates a positive modulatory role for the two antioxidants in alcohol-induced hypertension and associated renal dysfunction.

The assessment of creatinine and urea nitrogen levels has replaced the classically used inulin clearance by the kidneys in the measurement of glomerular filtration rate and accurate determination of the functional status of the kidneys.[49,50] From our results, a significant increase seen in creatinine/urea levels following alcohol exposure without antioxidant treatment may suggest a reduction in renal functional activity and has been reported as a common complication of acute kidney injury.[51] Under physiological conditions, 40–60% of filtered urea in the glomerular filtrate is conserved by reabsorption through the renal tubules, but this becomes significantly altered when the kidneys are not performing optimally.[52] Creatinine, produced from the breakdown of creatine and phosphocreatine, circulates in the blood, and is solely eliminated by the kidneys; thus, constituting renal function marker.[53,54] Therefore, observation of lower urea and creatinine levels in rats treated with alpha-tocopherol and quercetin and their combinations suggest potent protective effects for the two antioxidants especially when used in combination. This assertion is further strengthened by the absence of histopathologic lesions in the renal tissues of rats treated with alpha-tocopherol, quercetin, and their combination. Reports from this study corroborate earlier reports of Zal *et al*.,[55] who reported induction of lowered renal functional markers following administration of quercetin/Vitamin E cocktail to cyclosporine administered rats. Vitamin E is a chain breaking antioxidant, whose mechanism of action manifests during lipid peroxidation, with the hydroxyl group of the Vitamin E reacting with the peroxyl radical to form the corresponding lipid hydroperoxide and the chromanoxyl radical.[56] Quercetin has also been reported to improve renal function by reducing the levels of creatinine, BUN, and urinary uric acid in a rat model of adenine-induced chronic kidney disease.[57] Likewise, using chromium as the toxicant, quercetin in combination with N-acetylglucosamine exerted a dose-dependent efficacy demonstrated by a significant increase in animal survival, renal excretory function, and normalization of nitrogen metabolism in rats.[58] Moreover, alpha-tocopherol reportedly exerts an antiatherogenic effect by ameliorating oxidative processes, thereby reducing formation of atheromatous plaques, morbidity and mortality in renal failure patients.[59]

Furthermore, immunohistochemical evaluation revealed alcohol-induced increased expressions of cTn I in cardiac tissues of rats compared with those that received the antioxidants either singly or in combination, although with a significantly lowered expression of cTn I in the rats treated with the combination rather than individual treatment. The cTn I is a useful biomarker for the detection of other cardiac pathologies including acute coronary syndromes.[60] Accumulating evidence has indicated a unique role for inflammation in EtoH-induced organ damage, including cardiotoxicity.[61] Similarly, immunohistochemical staining of paraffin embedded renal tissues of rats revealed significant attenuation of ACE expression in alpha-tocopherol and quercetin administered rats compared with the rats exposed to only the toxic effects of alcohol. The ubiquitously distributed ACE stimulate the formation of an endogenous vasoconstrictor – angiotensin II. The observation of heightened expression of ACE in rats exposed to the toxic effects of alcohol without antioxidant treatment points to an intensification of Ang II formation from increased ACE activity with consequent hypertension. The QT interval on surface electrocardiogram represents the sum of depolarization and repolarization process of the ventricles,[62] and its prolongation, has been pathophysiologically linked with decreases in repolarizing outward potassium currents or increases in depolarizing inward sodium or calcium currents.[63] Various factors, including exposures to drugs and chemicals have been reported to prolong QT interval and increase predisposition to arrhythmia.[64] The observation of prolonged QTc in this study corroborate an earlier report of Li *et al*.[65] who linked heavy alcohol consumption with increased predisposition to prolonged QTc interval in human subjects. Therefore, normalization of the QTc interval in rats treated with alpha tocopherol and quercetin, in this study, strongly suggest potent cardioprotective effects for these antioxidants.

CONCLUSION

This study concludes that the oral administration of alpha-tocopherol and quercetin significantly ameliorated alcohol-mediated changes of primary hemodynamic parameters, electrocardiographic changes, antioxidant defense status, renal functional markers, histopathogic changes, and immunohistochemical expressions of cTn I and ACE.

Ethical approval

The research/study approved by the Institutional Review Board at University of Ibadan, number TOA/20/0036, dated April 5, 2020.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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