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Vitamin C as a potential ameliorating agent against hepatotoxicity among alcoholic abusers

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Abstract. – OBJECTIVE: Drug and substance abuse remains a major medical problem globally. Alcohol consumption, particularly heavy drinking, is an important risk factor for many health problems and is a major contributor to the global burden of disease. Vitamin C has proven to be defensive against toxic substances and provides antioxidant and cytoprotective activity to hepatocytes. The aim of this study was to investigate vitamin C as a potential ameliorating agent against hepatotoxicity among alcohol abusers.

PATIENTS AND METHODS: This study was a cross-sectional study that included eighty male hospitalized alcohol abusers and twenty healthy people as a control group. Alcohol abusers received standard treatment plus vitamin C. Total protein, albumin, total Bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and 8-hydroxhguanosine (8-OHdG) were investigated.

RESULTS: This study reported that, in the alcohol abuser group, there was a significant increase in the total protein, bilirubin, AST, ALT, ALP, TBARS, SOD and 8-OHdG; on the other hand, there was a significant decrease in albumin, GSH and CAT compared with the control group. The alcohol abuser group treated with vitamin C showed a significant decrease in total protein, bilirubin, AST, ALT, ALP, TBARS, SOD and 8-OHdG; on the other hand, there was a significant increase in albumin, GSH and CAT compared with the control group.

CONCLUSIONS: This study's findings suggest that alcohol abuse induces significant alterations in various hepatic biochemical parameters and oxidative stress and that vitamin C has a partial protective role in countering alcohol abuse-induced hepatotoxicity. Using vitamin C as an adjunctive supplement to standard treatment may be helpful in minimizing the toxic side effects of alcohol abuse.

Key Words:
Alcohol, Abusers, Vitamin C, Hepatotoxicity, Oxidative stress.

Introduction

The comprehensive list of contemporary worldwide critical public health risks includes substance misuse as a key concern. The number of substance abusers dependent on alcohol or illicit drugs is great[1,2]. In 2015, everyday excessive use of alcohol stood at 18.4%[3].

The term oxidative stress pertains to the occurrence of a misbalance between reactive oxygen species (ROS) production at the cellular level and the capability of the biological system to eliminate toxins deriving from those reactive productions[4,5]. Known as free radicals, these can be derived from endogenous as well as exogenous sources. Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress and the natural aging process can all drive endogenous free radical production, whilst exogenous free radical production often emerges due to the consumption of substances such as cyclosporine, gentamycin and bleomycin, foodstuffs including smoked meat, and cigarette smoke, alcohol or radiation[6,7].

A high rate of alcohol abuse has a significant negative impact on global health, particularly

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when alcohol consumption is extremely high and alcohol use is a major cause of disease. Around thirty critical health conditions are caused by alcohol misuse and a far bigger range of diseases are associated with alcohol as a key factor. Alcohol use is a major factor in the onset of infectious diseases, cancer, diabetes, neuropsychiatric diseases (such as alcohol use disorders), cardiovascular diseases, liver and pancreatic diseases and unintentional and intentional injuries[8].

Both acute and chronic use of ethanol (alcohol) will drive increased production of ROS, and this in turn drives the peroxidation of lipids, proteins and DNA across a range of systems and cells. Whilst the primary ethanol-induced alterations occur in the liver, extra hepatic alteration is also proven to exist[9].

Alcohol use affects metabolic processes in organs such as the brain, heart, kidneys and stomach. The deleterious impacts act metabolically via oxidative (acetaldehyde) and non-oxidative (ethyl esters of fatty acids) pathways. This produces increased free radical production and lipid peroxidation[3].

From a pathophysiological perspective on alcoholic liver disease (ALD) there is significant room for further research, but it seems clear that oxidative stress is a key contributor. It prompts and exacerbates the progression of liver damage. Ongoing exposure to alcohol provokes the microsomal ethanol-oxidizing system (MEOS), thus driving the process of the transformation of ethanol to acetaldehyde, thereby damaging various organs, most notably the liver[10,11]. MEOS induction boosts the production of ROS, leading to damage to hepatocytes as well as variations in gut microbiota, prompting the production of endotoxins and thereby spurring liver damage[12].

The overall degree of resilience to ROS is defined as the total antioxidant status. To counter the deleterious effects of free radical excesses, compounds that neutralize them can serve to protect organs. Both enzymes and non-enzymatic compounds can serve this purpose. Smaller non-enzymatic molecules such as vitamins A, E and C, as well as uric acid, cysteamine, pyruvate, hypotaurine and reduced glutathione, can all play a role, with the latters being highly abundant in the genital tract, in gametes and in embryos[13].

Ascorbic acid (vitamin C) constitutes a water-soluble compound which is included in the wider category of natural antioxidants. It can protect against oxidative stress on various tissues. In terms of its antioxidant functions, ascorbate scavenges oxygen or nitrogen-based radical species generated throughout normal cellular metabolism[14].

A range of clinical studies[15] has ascertained that vitamin C is a major protector against a number of diseases caused by oxidative stress, including cardiovascular disease, cancer and cirrhosis. Vitamin C serves as a superoxide scavenger in the primary hypertension process to eliminate symptoms among patients.

Alcohol use disorder can induce vitamin C deficiency by various mechanisms such as malabsorption, malnutrition, increased urine excretion of vitamin C produced by alcohol. Alcohol abusers with untreated vitamin C deficiency risk developing severe consequences and symptoms of alcoholic liver disease[16].

Malnutrition and unfavorable living situations can result from alcohol use disorder and lead to various vitamin deficiencies, mainly vitamin B1, B3, and C deficits. Vitamin C insufficiency was found[17] to occur more frequently than vitamin B1 deficiency. However, there has not been much follow-up on these important discoveries, which could have a big impact on the chronic alcoholic's detox.

Alcohol may cause a toxic effect on enterocyte that may lead to malabsorption of vitamins while hepatotoxicity induced by alcohol prevents enough vitamins from being converted to their active metabolites in the liver[18].

Some scholars[19,20] have found that vitamins C and E can protect patients from liver disease caused by alcohol or dysfunction and can attenuate lipid peroxidation injury in rats. This means they may be more effective in combination than when consumed on their own, when addressing alcohol-mediated toxic effects during liver regeneration. Due to these ameliorating effects of vitamin C on the alteration of redox status and because no human studies are to date evident in the literature, the present study is on the ameliorating effect of vitamin C on the alteration of redox status in alcohol abusing patients[21]. This study aims to investigate vitamin C as a potential ameliorating agent against hepatotoxicity among alcohol abusers.

Patients and Methods

Subjects

The present study was a cross-sectional study conducted at the PRC (Al Amal Hospital for Mental Health) in the Aseer region of the Kingdom of Saudi Arabia (KSA). The sample consists of eighty male

alcohol abusers and twenty healthy individuals used as a control group. The study was conducted in compliance with credible research protocol, sound clinical practice and the criteria for clinical studies set by the Saudi Food and Drug Administration. The study was open-label and was conducted over a period of a single year. The participants in the experimental group were all hospitalized patients undergoing treatment for alcohol abuse. Their medical histories were recorded, and they had been interviewed by an experienced psychiatrist and psychiatrically assessed in accordance with the DSM-5 criteria. All patients received their standard treatments plus an administration of vitamin C. The vitamin C dose was ingested in the form of an effervescent tablet, at a dosage of 1,000 mg/day over 7 days[22]. Vitamin C was administered under the supervision of the clinical supervisor at 8:00 am daily, alongside the standard treatments for alcohol abuse. The clinical supervisor monitored patients daily to observe any adverse effects from the treatment. Follow-up reviews of the patients were conducted one month after treatment.

The participants' general medical histories, including smoking status and substance use disorder (SUD) status (including duration of abuse, age of participants, work environment, marital status, education levels and types of substance used) were all obtained. Inclusion and exclusion criteria were applied. All patients were positive to alcohol abuse with impaired liver function test and treated under the same regimen. Whereas the exclusion criteria included patients who consumed antioxidant, liver disease and received any medication that might affect the measured biochemical parameters.

Medical Examination

Histories of patients were obtained from official medical records, covering age, sex, lifestyle habits, occupation, toxicological history, diseases and drug treatments. Complete physical examinations were performed. The height and weight of each participant were measured to calculate their body mass index (BMI).

Study Approach

Information regarding the participants was collected using a pre-designed three-section questionnaire. The first section consisted of questions pertaining to personal and socio-demographic factors, including age, marital status and lifestyle habits such as smoking and socializing. The second section comprised of questions pertaining to abuse history, such as reasons for abuse, motives for alcohol consumption, the duration of abuse and the type of SUD. The third section asked about past medical problems.

Blood Sample Collection and Storage

Blood samples were collected under aseptic conditions via clean venepuncture using sterile disposable syringes. Approximately 10 ml of blood was taken from each participant as well as from the control sample. The blood was placed into a clean and dry silicon-coated test-tubes and allowed to clot at room temperature for 20 minutes. The blood was centrifuged at 2,000 rpm for 10 minutes and the supernatant was aliquoted into 1.7 ml eppendorf tubes. Serum samples were then stored in tightly closed vials at -80°C until used for analysis. The samples were collected both at the admission stage and after the detoxification period.

Blood Alcohol Concentration

The main method for blood ethyl alcohol concentration (BEC) determination involves obtaining two tubes of venous blood immediately after the arrival of the patients. Benzalkonium chloride was hereby used for cleansing the venepuncture site, and paired samples were then drawn into plain blood collection tubes. The time of collection was recorded in the data management system. Samples were then delivered straight away to the emergency laboratory and centrifuged at 3,000 xg for 10 minutes after delivery. Measurements were conducted for both specimens straight after centrifugation. Blood ethanol concentration was analyzed using a Synchron Systems Ethanol Assay kit (A-E 474947) by employing an enzymatic rate method on the Beckman-Coulter Olympus AU400 auto analyzer (Beckman Coulter Inc., Melville, NY, USA). In this process, alcohol dehydrogenase catalyzes the reaction of ethanol and nicotinamide adenine dinucleotide (NADH) to acetaldehyde and NADH. The rate of change in absorbance at 340 nm was recorded to determine the ethanol concentration in the sample. The Synchron Systems Ethanol Assay kits' information sheet reports an analytical measurement range of 0.05 to 6.00 g/L, with a lower limit of quantification of 0.04 g/L and a precision between 1.3% and 2.6%[23].

The Clinical Institute Withdrawal Assessment of Alcohol Scale (CIWA-Ar)

The Clinical Institute Withdrawal Assessment of Alcohol Scale revised is a 10-item questionnaire

that measures the current degree of severity of an individual's alcohol withdrawal symptoms[24].

Liver Function Tests

The biuret reaction was used to determine total serum protein as per Gornall et al[25]. Serum albumin concentration was measured according to the method developed by Bowers and Wong[26], using a commercial kit supplied by Diamond (Cairo, Egypt). The approach used here was that used by Walter and Gerade[27]. A kit supplied by Diamond (Cairo, Egypt) was deployed in order to estimate the serum activity of AST following the approach used by Reitman and Frankel[28]. This approach was used here to measure the levels of serum ALT, using a commercial kit supplied by Diamond (Cairo, Egypt). The serum level of ALP was determined using a commercially available kit (BioMérieux Co, Marcy-l'Étoile, France) and adopting the method used by Belfield and Goldberg[29].

Biomarkers of Oxidative Stress

The human thiobarbituric acid reactive substance (TBARS) ELISA kit was used for quantitative measurement. The thiobarbituric acid reactive substance (TBARS) ELISA kit was purchased from MyBiosource, Inc. (San Diego, CA, USA) (catalogue number MBS166987). The human reduced glutathione (GSH) ELISA kit was used for quantitative measurement. This was bought from MyBiosource, Inc. (San Diego, CA, USA) (catalogue number MBS727656). The human superoxide dismutase (SOD) ELISA kit was used for this quantitative measurement. This was sourced from MyBiosource, Inc. (San Diego, CA, USA) (catalogue number MBS2707322). The human catalase (CAT) ELISA kit was used for this quantitative measurement. It was sourced from MyBiosource, Inc. (San Diego, CA, USA) (catalogue number MBS165657). The human 8-Hydroxy-desoxyguanosine (8-OHdG) ELISA kit was used for this quantitative measurement. This was sourced from MyBiosource, Inc. (San Diego, CA, USA) (catalogue number MBS160699).

Measured Outcomes

The primary outcome of our study was chronic liver injury including grade 1 and 2. The secondary outcomes of our study were improvement in the values of liver enzymes.

Statistical Analysis

Data were collected, tabulated and analysed by Paired samples *t*-tests to discern significant differences in the data pertaining to the participants from both before and after the administration of Vitamin C. Results were expressed as means ± SD and were considered statistically significant if the *p*-value was lower than 0.05. Pearson Correlation's coefficients were used to establish the relationships between duration of addiction, alcohol levels, oxidative stress biomarkers and hepatic parameters. SPSS Version 21.0 (IBM Corp., Armonk, NY, USA) was used to conduct the statistical analyses.

Results

Sociodemographic Characteristics

The ages of participants ranged from 18 to over 45 years, with mean ages of 28.30 ± 4.70 and 28.00 ± 6.80 in group I and group II respectively. No significant difference between group I and group II occurred in terms of mean age ($p > 0.05$). Table I shows how the majority of patients were aged between 26 and 35 years old. This group formed 65.00% and 48.75% of group I and group II respectively. It highlights how the majority of the participants came from urban areas in both group I and group II (80.00% and 83.75% respectively). In terms of distribution of participants according to marital status, the majority of participants were single in both groups I and II (55.00% and 72.50% respectively). Most participants were living with their friends (70.00% and 36.25% in group I and group II respectively). In terms of distribution of participants according to levels of educational attainment, the highest percentages of patients had a higher-level education (85.00%), with the lowest percentage groups being patients who were non-educated or postgraduates (1.25% and 2.50% respectively).

Duration of Alcohol Use

As per Table II, duration of alcohol intake for the sample as a whole ranged from 2 to 5+ years, with a mean duration of 5.40 ± 1.60 years in group II.

Motivation for Alcohol Use

Regarding the motives the patients expressed for alcohol consumption, lifestyle stress and peer pressure (influence of friends) comprised the biggest drivers for starting and continuing alcohol intake, at 37.5% and 33.75% respectively (as per Table III).

Table I. Distribution of the control and addict groups (n=100) according to their ages, residence, marital status, social status, educational levels, occupational status and special habits.

Items		Group I (n=30)	Group II (n=30)
Age (years)	Mean ± SD	28.30±4.70	28.00±6.80
Residence (%)	Urban	80	83.75
	Rural	20	16.25
Marital status (%)	Single	55	72.50
	Married	30	20
	Divorced	15	7.50
Living status (%)	Living with family	Nil	28.75
	Living alone	30	35
	Living with friends	70	36.25
Educational levels (%)	Non-educated	10	1.25
	High school	65	85
	Graduate	25	11.25
	Postgraduate	Nil	2.50
Occupational status (%)	Student	Nil	12.50
	Employee	65	32.50
	Unemployed	35	55
Special habits (%)	Smoker	100	100
	Caffeine user	100	100

Group I – Control group; Group II - Alcohol abuser group.

Table II. Distribution of the Alcohol abuser group (n=80) according to the duration of alcohol intake (in years).

Duration of intake (years)	Group II (n=80)		
	No.	%	Mean ± SD
2 - 3 years	6	7.50%	
4 - 5 years	44	55.00%	5.40±1.60
More than 5 years	30	37.50%	

Group II – Alcohol abuser group.

Table III. Distribution of alcohol abuser group (n=80) according to motives for alcohol intake.

Motives for substance intake	Group II (n=80)	
	No.	%
Lifestyle stress	30	37.50%
Influence of friends	27	33.75%
Frustration	13	16.25%
Relationship difficulties	10	12.50%

Group II – Alcohol abuser group.

Table IV. Alcohol level and Clinical Institute Withdrawal Assessment of Alcohol Scale (CIWA-Ar) for alcohol abuser group (n=80) prior to treatment.

Items	Group II (n=80)
Alcohol level (mg/dl)	119.60±41.47
CIWA-Ar	40.10±4.21

Group II – Alcohol abuser group.

Alcohol Level and Severity Clinical Institute Withdrawal Assessment of Alcohol Scale

The mean alcohol level was 119.60 ± 41.47 mg/dl. The severity Clinical Institute Withdrawal Assessment of alcohol scale values stood at 40.10 ± 4.21 (as per Table IV).

Body Mass Index

Figure 1 shows that there was no significant change in mean body mass index values for groups IIB (before treatment) and IIA (after treatment) compared to the control group ($p < 0.05$). Mean body mass index showed no significant change in group IIA when compared to group IIB ($p < 0.05$).

Liver Function Tests

Figure 1 shows a significant increase in mean total protein, total bilirubin, aspartate aminotransferase activities, alanine aminotransferase activities and alkaline phosphatase activities values in groups IIB and IIA when compared with the control group ($p < 0.05$). Mean total protein, total bilirubin, aspartate aminotransferase activities, alanine aminotransferase activities and alkaline phosphatase activities showed a significant increase in group IIA when compared to group IIB ($p < 0.05$).

As evident in Figure 1, a significant decrease emerged in the mean albumin values for group IIB when compared to the control group ($p < 0.05$). Mean albumin values were significantly

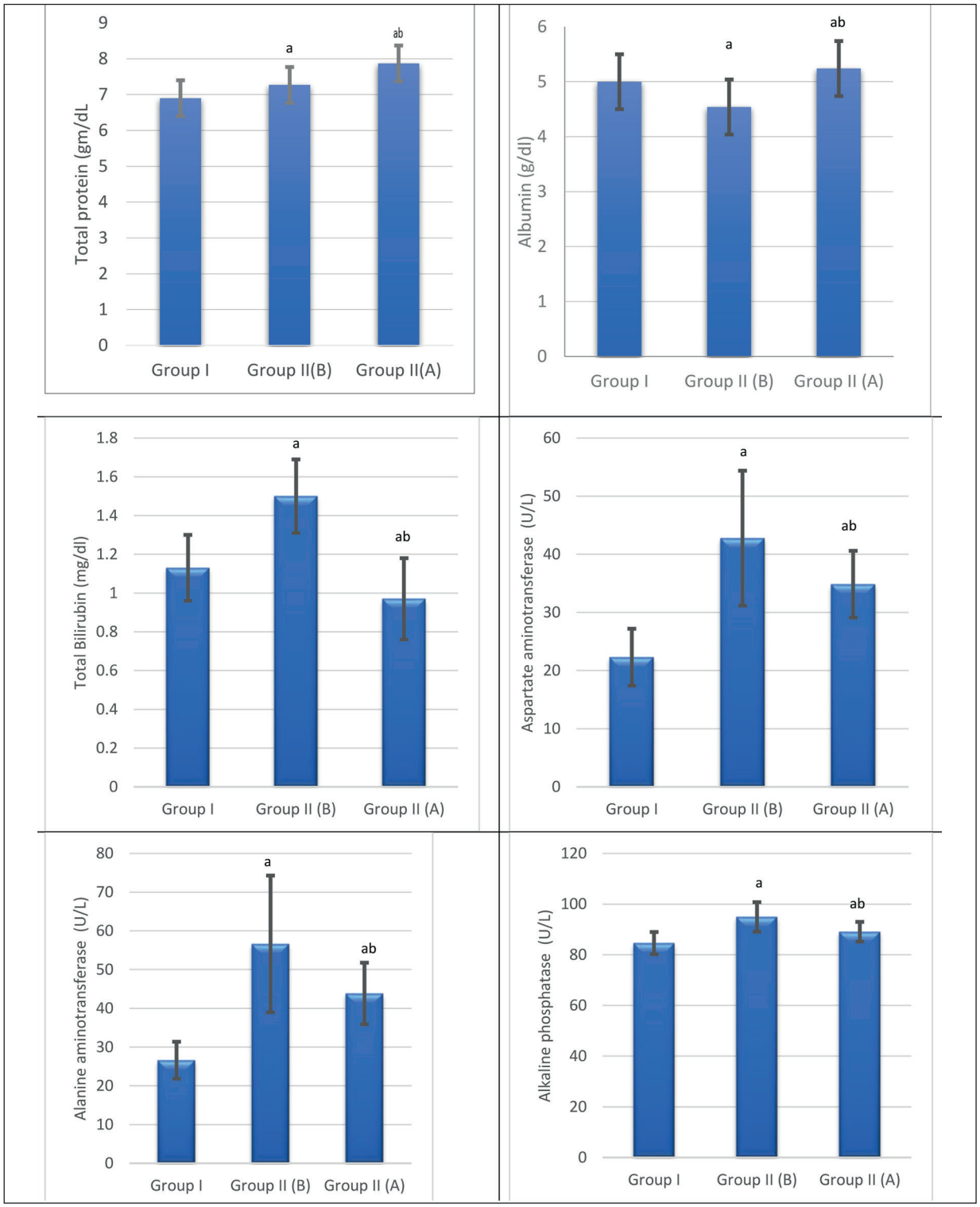

Figure 1. Mean total protein, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase values for the control and alcohol abuser groups (n=100) before treatment (group IIB) and after treatment (group IIA). Value was significantly different from [a]control and [b]before treatment groups at $p \leq 0.05$.

increased in group IIA when compared to the control group ($p < 0.05$). The mean albumin values showed a significant increase in group IIA compared to group IIB ($p < 0.05$).

Serum Oxidative Stress Biomarker Levels

As per Figure 2, there was a significant increase in mean thiobarbituric acid reactive substances, mean superoxide dismutase and 8-hydroxhguanosine values for group IIB and no signficant change in group IIA compared to

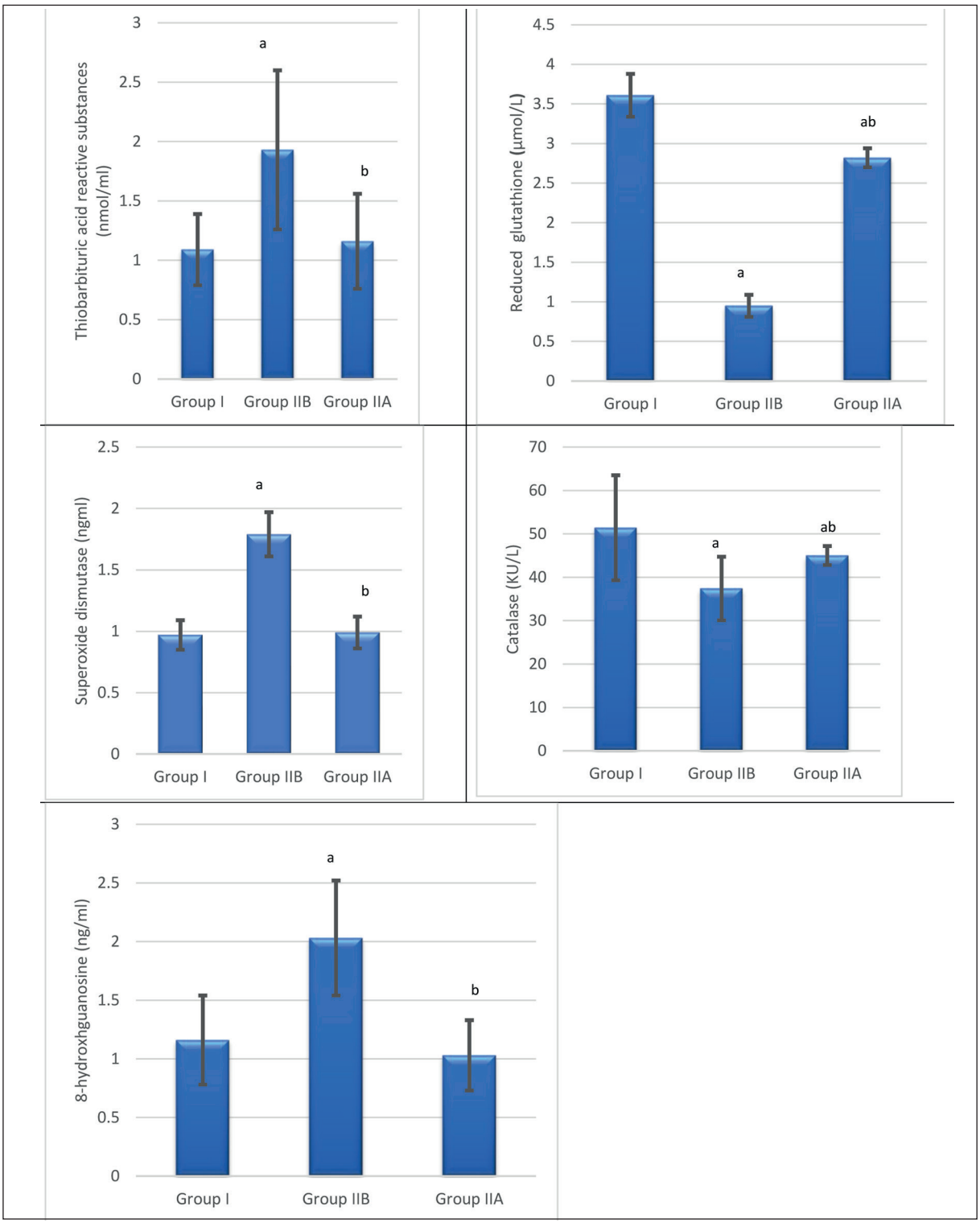

Figure 2. Mean thiobarbituric acid reactive substances, reduced glutathione, superoxide dismutase, catalase and 8-hydroxhguanosine values for the control group and alcohol abuser group (n=100) before treatment (group IIB) and after treatment (group IIA). Value was significantly different from [a]control and [b]before treatment groups at $p \leq 0.05$.

the control group ($p < 0.05$). Mean thiobarbituric acid reactive substances, mean superoxide dismutase and 8-hydroxhguanosine showed a significant decrease in group IIA when compared to group IIB ($p < 0.05$).

Figure 2 shows that there was a significant decrease in mean reduced glutathione values in groups IIB and IIA as compared to the control group ($p < 0.05$). The mean reduced glutathione showed a significant increase in group IIA compared to group IIB ($p < 0.05$).

As per Figure 2, a significant decrease was found in mean catalase values for groups IIB and IIA when compared with the control group ($p < 0.05$). Mean catalase showed a significant increase in group IIA compared to group IIB ($p < 0.05$).

Correlations of the Studied Parameters

Liver function tests

Table V shows that total protein had no significant correlation with CIWA-Ar, alcohol level and age before and after treatment, but it has a negative significant correlation with duration of alcohol intake before and after treatment. The Table also shows that albumin had no significant correlation with CIWA-Ar and alcohol level before treatment, but there is a negative significant correlation with the duration of alcohol intake before treatment. It had no significant correlation with the duration of alcohol intake after treatment. Table V also shows that total bilirubin had no significant correlation with CIWA-Ar, alcohol level, age and duration of alcohol intake before and after treatment. Aspartate aminotransferase had no significant correlation with alcohol level and age before and after treatment, but a positive significant correlation with CIWA-Ar before treatment is evident. There was no significant correlation with CIWA-Ar after treatment. There were positive significant correlations with duration of alcohol intake before and after treatment. From Table V, we can see that alanine aminotransferase had no significant correlation with alcohol level, age and duration of alcohol intake before and after treatment, but it did have a positive significant correlation with CIWA-Ar before and after treatment. It is also clear that alkaline phosphatase had no significant correlation with alcohol level, age and duration of alcohol intake before and after treatment, but there was a positive significant correlation with CIWA-Ar before treatment. There was no significant correlation after treatment.

Serum oxidative stress biomarker levels

Table VI shows that thiobarbituric acid reactive substances had no significant correlation with CIWA-Ar and age before and after treatment, but it shows a positive significant correlation with alcohol level and duration of alcohol intake before and after treatment. The Table also shows that reduced glutathione had no significant correlation with CIWA-Ar before and after treatment. It shows a negative significant correlation with alcohol level before treatment and no significant correlation after treatment. It shows a negative significant correlation with age before and after treatment. There was also negative significant correlation with duration of alcohol intake before treatment and no significant correlation after treatment. Table VI also shows that superoxide dismutase had no significant correlation with CIWA-Ar and age before and after treatment. On the other hand, a negative significant correlation with alcohol level and duration of alcohol intake before treatment was evident. There was no significant correlation with alcohol level and duration of alcohol intake after treatment. The Table shows that catalase had no significant correlation with alcohol level and age before and after treatment. It showed negative significant correlation with CIWA-Ar and duration of alcohol intake before treatment. On the other hand, it showed no significant correlation with CIWA-Ar and duration of alcohol intake after treatment. From Table VI we can see that 8-hydroxhguanosine had no significant correlation with CIWA-Ar and age before and after treatment. It had no significant correlation with alcohol level before treatment and a positive significant correlation after treatment. It showed a positive significant correlation with duration of alcohol intake before treatment but no significant correlation after treatment.

Discussion

The study had no female volunteers mainly because of important socio-cultural gender norms pertaining to case privacy. This phenomenon has been recently documented[4] in the Qassim region. One study[30] has found that the percentage of female SUD patients in Riyadh stands at roughly 12%, but studies[31,32] have flagged up the major

Table V. Pearson correlation's coefficients between CIWA-Ar, alcohol level, age, duration of alcohol intake, and liver function test values in alcohol abuser group before treatment (IIB) and after treatment (IIA).

	CIWA-Ar		Alcohol level		Age		Duration of alcohol intake	
Items	**IIB**	**IIA**	**IIB**	**IIA**	**IIB**	**IIA**	**IIB**	**IIA**
Total protein (g/dl)	-0.025	-0.151	0.030	0.108	-0.119	-0.008	-0.338**	-0.221**
Albumin (g/dl)	-0.086	-0.185	-0.141	0.074	-0.119	0.117	-0.251*	-0.066
Total Bilirubin (mg/dl)	0.013	0.053	-0.139	-0.169	-0.018	0.111	-0.048	0.076
AST (U/L)	0.419**	0.147	0.093	-0.079	-0.120	-0.067	0.309**	0.319**
ALT (U/L)	0.478**	0.320**	0.046	-0.011	0.010	0.094	0.145	0.167
ALP (U/L)	0.279*	0.019	0.074	-0.036	0.109	0.183	0.139	0.137

Group I – IIB – Before Treatment; IIA – After Treatment. AST – Aspartate Aminotransferase; ALT – Alanine Aminotransferase; ALP – Alkaline Phosphatase. *Correlation was statistically significant at 0.05 level (2-tailed). **Correlation was statistically significant at 0.01 level (2-tailed).

challenges in assessing SUD in women. Males have been found[33] to show a significant risk of developing drug use disorders in Saudi Arabia. This is partly explained by the fact that males have greater freedom at their disposal than females, including the freedoms to go out and stay out late into the night, as well as the freedom of general mobility in public places and the freedom to holiday with peers.

Several sociological factors have driven alcohol consumption levels up. The age group most affected by alcohol use problems is the 26-45 group. Alcoholic patients in one study[34] ranged in age from 20 to 36 years old, with the mean age range being 32-36 years. A global survey[35] has shown that alcohol misuse among younger people is on the rise, with age groups affected ranging from 18 to 54 years.

The marital status of alcohol abusers tended to be single in the present study. Single marital status has also been linked to alcoholism in other reports[36]. Marriage is linked to a significant reduction in the probability of developing an alcohol use disorder. This former research[36] confirms the present study's findings.

There is a clear link between alcohol abuse and friendship according to the wider body of research[37]. This study discerned the same link. Most of the patients in this study had a higher-level education. Alcoholics with a higher-level education who have encountered failures in success are posited to be at high risk[38]. The study[39] also found evidence of significant links between employment status and the likelihood of consuming alcohol at high levels.

The etiological and reasons-for-drinking literature to date focuses on two main drivers of alcohol consumption, whereby (a) people drink to deal with stress or (b) people drink due to societal and peer pressure. A strong association is evident between drinking to deal with stress and perceived stress, as well as between drinking for social reasons and the alcohol intake levels of peers[40]. The most critical driver of the onset of alcohol consumption disorder, according to the present study, is life-induced stress.

Alcohol consumption can often induce ALD. The quantity and the type of alcohol consumed are the vital risk factors in the development of ALD. ALD onsets in roughly 90% of cases of

Table VI. Pearson correlation's coefficients between CIWA-Ar, alcohol level, age, duration of alcohol intake, enzymatic antioxidants and lipid peroxidation in alcohol group before treatment (IIB) and after treatment (IIA).

	CIWA-Ar		Alcohol level		Age		Duration of alcohol intake	
Items	**IIB**	**IIA**	**IIB**	**IIA**	**IIB**	**IIA**	**IIB**	**IIA**
TBARS (nmol/ml)	0.036	0.092	0.569**	0.226*	0.145	0.119	0.459**	0.422**
GSH (ng/ml)	-0.116	-0.102	-0.726**	-0.105	-0.667**	-0.267*	-0.562**	-0.121
SOD (U/ml)	-0.036	-0.116	- 0.628**	-0.116	-0.050	-0.065	-0.812**	-0.062
CAT (ku/l)	-0.252*	-0.192	-0.172	-0.006	-0.191	-0.077	-0.504**	-0.107
8-OHdG (ng/ml)	0.206	0.156	0.113	0.227*	0.034	0.066	0.226*	0.062

IIB – Before Treatment; IIA – After Treatment. TBARS – Thiobarbituric Acid Reactive Substances; GSH – Reduced Glutathione; SOD – Superoxide Dismutase; CAT – Catalase; 8-OHdG – 8-hydroxhguanosine. *Correlation was statistically significant at 0.05 level (2-tailed). **Correlation was statistically significant at 0.01 level (2-tailed).

people who consume alcohol and develop alcoholic hepatitis. Aside clinical histories of chronic alcoholism, estimations of ALT and AST are standard laboratory investigations for aminotransferases, reflecting damage to hepatocytes41.

The marked increases in serum AST, ALT and ALP activities in patients with alcohol use disorder found here align with previous study findings[42]. The biggest etiological factor suggested is ethanol-induced oxidative stress, caused by combined antioxidant defense mechanism impairment and reactive oxygen species production by the mitochondrial electron transport chain, the alcohol-inducible cytochrome P450 (CYP) 2E1 and activated phagocytes[43].

The present study found that the presence of an alcohol use disorder increases the activity of liver enzymes (AST, ALT and ALP) and increases the levels of total protein, which have been linked[44] with oxidative stress defense systems.

Data so far show higher levels of serum albumin in people with alcohol use disorders, indicating higher rates of albumin protein synthesis as response to regular ethanol intake prior to the onset of liver dysfunction. Rates of hepatic protein synthesis are evidently lower in people with advanced liver disease. The specific processes behind this finding are not known, but former studies[45] of cell cultures have found that continuous ethanol consumption causes increased hepatic protein production rates.

Former research[46] highlighted that smoking has a major effect on the activity of liver enzymes. All participants in this research were smokers so no meaningful comparative data could be collected. The effects of smoking have thus been removed from the study's analyses.

To increase the discriminative potential of biomarkers indicating liver conditions, normal ranges could be redefined using databases pertaining to healthy people who do not drink alcohol. The potential functions of a wide range of biochemical indicators as actors in oxidative stress defense systems, moreover, require ongoing research. Vitamin C (as an antioxidant) was found here to reduce ethanol-induced increases in AST and ALT.

Vitamin C's antioxidant properties may explain this, because they may have maintained free radicals in alcohol use disorder patients at lower levels, reducing the oxidative challenges that hepatic cell membranes (and possibly other cells) are exposed to, thereby decreasing damage to liver cells[47].

Vitamin C appeared to control the intensity of hepatic function parameters as well as blood hydroperoxide values in hepatic cells. It may protect cell integrity and boost the effects of alanine aminotransferase. Treatment with vitamin C (1,000 mg/day) had contributed to the normalization process and shifted the above-mentioned parameters from extremely high levels to normal reference ranges. Treating with vitamins C people with liver injuries caused by alcohol use (and driven by oxidative damage) produced notable reductions in blood ALT, AST and ALP levels, according to the present study. Chronic ethanol exposure serves to increase the production of ROS, lower cellular antioxidant levels and enhance oxidative stress in many tissues, especially in the liver and causes liver injury[48].

The TBARS concentration levels reflect the level of malondialdegyde (MDA), which is the end product of lipid peroxidation highlighted by Peng et al[49]. The MDA concentration found here not only significantly increased in the alcohol group compared to the control group, but it was also significantly correlated with the duration of alcohol dependence. The present study identified significantly increased levels of TBARS compared with the controls. Peng et al[49] and Gupta et al[50] have produced similar findings. Because MDA is a known biomarker of oxidative stress, a range of animal and human studies[51] indicate the possibility of oxidative stress in the pathogenesis of alcoholic liver disease arising from the excessive production of alcohol-mediate ROS.

GSH has a key role in the detoxification of xenobiotics and in the maintenance of the redox status of cells. A decline in GSH levels is thought to be indicative of oxidative stress. The decrease found[52] in this endogenous antioxidant is connected with ethanol-induced oxidative stress, which involves the generation of toxic acetadehyde and other reactive molecules, as assessed by the findings of another key study[53]. In this study, GSH was significantly lower in the alcohol use disorder group than in the control group. This lower GSH level may be because of impaired biosynthesis or decreased release from damaged liver cells. This study showed that alcohol use disorder decreases the level of GSH in subjects. These findings align with those of Loguercio et al[54].

The antioxidant enzymes GSH, CAT and SOD all protect the body against oxidative stress[55]. Both increased and decreased SOD activities in the blood of alcoholics have been reported here[56]. This could be because of different durations of

alcohol dependence. Free radical scavenging enzymes such as SOD, CAT and GSH are known to be the first line of cellular defense against oxidative damage, disposing of superoxide anion and H_2O_2 before they interact to form the more harmful hydroxyl radical.

In this study, both CAT and SOD activities decreased significantly in the alcohol group as compared to the control group. It is thought[57] that excessive superoxide anions may prompt lipoperoxide formation and induce cell damage before being converted to H_2O_2 by SOD. Given there is no adequate SOD activity, the superoxide anion is not converted into H_2O_2, which is the substrate for the H_2O_2 scavenging enzyme CAT. There is therefore an inactivation of the H_2O_2 scavenging enzyme CAT, leading to a decrease in its activity[58].

Vitamin C is a water-soluble vitamin, acting as a physiological antioxidant that protects cells against diseases caused by oxidative stress. Vitamin C has interactive effects which can reduce cellular damage caused by ROS. It is also used to neutralize oxidative damage in patients with alcohol use disorder. It serves to scrub free radicals and neutralize oxidative stress[59].

The chemical structure of vitamin C, comprised of hydroxyl groups attached to a furan ring, suggests that it is a relatively easy donor of electrons and protons. It can thus play a role in redox reactions. Vitamin C can be oxidized simultaneously, reducing other ROS compounds, in particular the superoxide anion radical. Competitive reactions of vitamin C with ROS scavenge them before they reach various cell components. Vitamin C is hence oxidized instead of lipid membranes, proteins and DNA, protecting these structures from damage. Oxidation transforms vitamin C into dehydroascorbic acid. This can then be enzymatically converted back to vitamin C using glutathione[60].

Vitamin C not only scavenges ROS but also inhibits their formation. The inhibition of oxidative damage to erythrocyte membranes' lipids has been observed in a key study by Yang et al[61] who found a decrease in phosphatidylcholine hydroperoxide content (markers of lipid peroxidation), and another study by Candan et al[62] who found a decrease in TBARS concentration following oral vitamin C supplementation. The present study found that mean TBARS concentration decreased significantly in the vitamin C-treated group.

Vitamin C was found to reduce superoxide anions via the modulation of antioxidant enzyme (SOD) activity[63]. This aligns with the findings of Rehman et al[64] who found an elevated level of SOD in mice after vitamin C supplementation.

Vitamin C also increases the activity of catalase (CAT). Patra and Swarup[65] found that the administration of vitamin C significantly inhibited lipid peroxidation levels and increased catalase (CAT) levels in rats. These finding align with those of the present study.

The study also found that vitamin C supplementation has a protective role against oxidative DNA damage. Human studies of the role of vitamin C in protecting against oxidatively induced DNA damage are to date ambiguous in terms of validity and show inconsistent results. Several researchers[66] have found a reduction in ex vivo or in vivo DNA oxidation after vitamin C supplementation in healthy volunteers, but some have found[67] no change occurring. Others[68] have produced mixed results with a decrease in certain types of oxidized DNA bases but increases in other types.

Limitations of the Study

The present study has not investigated the possible confounding effects of gender or morphological patterns of liver histopathology. The types of beverage consumed by patients is another vital factor not considered here, and it could have influenced the results of the study. The duration of vitamin C treatment was brief and analyses of longer-term treatments would be valuable.

Conclusions

This study's findings suggest that alcohol abuse induces significant alterations in various hepatic biochemical parameters and oxidative stress, and that vitamin C has a partial protective role in countering alcohol abuse-induced hepatotoxicity. Using vitamin C as an adjunctive supplement to standard treatment may be helpful in minimizing the toxic side-effects of alcohol abuse.

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Ethics Approval

Approval for conducting the present study was obtained from the College of Pharmacy, Qassim University Ethics Committee, the Research Ethics Committee of the Asser re-

gion, and the Ministry of Health for Saudi Arabia. Assessments were conducted according to set protocols for human studies established by the Ethical Committee of the College of Pharmacy, Qassim University and the Research Ethics Committee at the Ministry of Health for Saudi Arabia (The ethical number was REC-06-10-2020 and SFDA approval number SCTR number was 20111803).

Informed Consent

Informed consent was obtained from all participants included in the study.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Misfer Hussain Al Garea, Ashraf Mahmoud Emara and Abdulmajeed A Alqasoumi contributed equally in all steps of the study including research design, analysis and interpretation of data, practical part, drafted the paper, revised it critically. Saad Ali Alqahtani and Ali Hadi Hadadi shared practical part of this study. All authors read and approved the final version of the manuscript.

Availability of Data and Materials

The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

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