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Protective effect of vitamin C against alcohol induced lungs toxicity in adult male Wistar rats

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Abstract. *Oyesola OA, Osonuga IO, George ET. 2023. Protective effect of vitamin C against alcohol induced lungs toxicity in adult male Wistar rats. Cell Biol Dev 7: 35-40.* The lungs are the major organs in the respiratory system. The toxic effect of alcohol on the lungs leads to cell death and loss of function. This study aimed to investigate the protective effects of vitamin C on alcohol-induced lung toxicity in male Wistar rats. Forty male Wistar rats were acclimated for 14 days and randomly divided into eight groups. Group A was the control and received only distilled water. Group B was given alcohol, while groups C, D, and E received varying doses of vitamin C. Groups F, G, and H received alcohol, followed by vitamin C. After 21 days of treatment, the rats' lungs were collected and evaluated for various parameters, including antioxidant enzyme activity (Catalase (CAT), Glutathione (GSH), and Superoxide Dismutase (SOD), lipid peroxidation levels (malodialdehyde (MDA), carbon dioxide (CO_2) levels in the blood, and histopathological changes. The results indicated that rats that received only alcohol had increased lipid peroxidation (MDA) levels, reduced antioxidant enzyme activity (CAT, SOD, and GSH) in the lungs, high CO_2 levels in the blood, dilation of the alveolar sac, and disorientation of the bronchioles. However, groups treated with alcohol and vitamin C exhibited increased antioxidant enzyme activity, reduced lipid peroxidation and CO_2 content of the blood, regenerative changes, and improvement in the histo-architecture of the lungs. Vitamin C has demonstrated protective properties against alcohol-induced lung toxicity.

Keywords: Antioxidants, carbon dioxide, lung, vitamins

INTRODUCTION

Alcohol consumption is a widespread global practise, and the effect of its consumption depends on the quantity and quality of alcohol consumed. Studies have suggested that moderate consumption of alcohol may reduce the risk of developing cardiovascular diseases (Rehm et al. 2017); other research has also found no clear relationship between the level of alcohol consumption and the cardiovascular system (Weng and Dunn 2019). However, the consumption of alcohol is related to the development of various diseases such as cancer, liver diseases, cardiovascular diseases, diabetes, and neuropsychiatric diseases (Rungratanawanich et al. 2021). The development of these above-mentioned diseases may be determined by genetic predisposition, malnutrition, and concurrent viral infection of the liver (Neuman et al. 2014; Teschke 2019).

Vitamins are important for various physiological and biochemical processes in the human body (Wishart 2019). Human beings need to get vitamins from external sources, such as diet, since they can't synthesise them (Capozzi et al. 2012). Vitamin C is an important water-soluble compound that is essential for many biological processes, including tissue repair, collagen formation, immune system function, increasing antioxidant enzyme activity, and the production and functioning of several enzymes (Lykkesfeldt and Tveden-Nyborg 2019). Vitamin C also plays an important role in enzymatic reactions, the manufacture of hormones and neurotransmitters, and other biological processes.

The lungs are important organs of the respiratory system that allow gas exchange between the human body and the external environment (Molnar and Gair 2013). Excessive consumption of alcohol can lead to the development of respiratory diseases such as Acute Respiratory Distress Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD), and pneumonia (Szabo and Saha 2015). Although the liver is responsible for the metabolism of ingested alcohol, a small percentage of the ingested alcohol travels through the bronchial circulation to the airway passage, where it undergoes oxidative and non-oxidative processes. Some alcohol may be expelled unaltered through breathing. The consumption of alcohol leads to weakness of the systemic immune system, making alcohol drinkers more vulnerable to lung infections with severe symptoms and unfavorable consequences such as ARDS and COPD (Kaphalia and Calhoun 2013). The consumption of alcohol may also lead to a deficiency of vitamin C. As mentioned above, vitamin C has antioxidant activity, which counters the oxidative stress induced by alcohol. It also has the ability to improve the production of collagen, a structural protein that supports the lungs and is vital in maintaining the elasticity of the lungs to function normally. Previous studies have shown that vitamin C has the ability to reduce the risk of respiratory infection by enhancing the immune activity of T cells and phagocytes, which fight off infections.

Additionally, vitamin C has been found to improve lung function and reduce airway inflammation in patients with asthma, a chronic respiratory disease characterized by airway inflammation and constriction (Schloss et al. 2020). This present study will evaluate the antioxidant and anti-inflammatory activity of vitamin C against alcohol toxicity in adult male Wistar rats.

MATERIALS AND METHODS

Animal care and grouping

For this experiment, forty (40) adult healthy Wistar male rats weighing 150g to 250g were utilized. The rats were housed in wire and plastic cages in the Olabisi Onabanjo University animal house at the Obafemi Awolowo College of Health Sciences, Sagamu Campus, Ogun State, Nigeria. The rats were given two weeks to acclimatize; they were fed a standard pellet diet and given unrestricted access to water. The National Research Council's (2011) internationally recognized standard rules for the use of animals were followed in the handling and care of the animals.

Ethical approval for the use and care of laboratory animals was obtained from the Ethical Committee for Research of the Department of Physiology, Faculty of Basic Medical Science (FBMS), Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria, with approval number OOU/PHSECR/22/009.

Eight groups of five rats each were formed randomly from the rat population, and each group received treatments for 21 days. All treatement was administered through the oral route of administration using an oral gavage.

Group A: Distilled water only

Group B: 6000 mg/kg body weight of alcohol (30% v/v)

Group C: 100 mg/kg body weight of vitamin C

Group D: 200 mg/kg body weight of vitamin C

Group E: 300 mg/kg body weight of vitamin C

Group F: 6000 mg/kg body weight of alcohol (30%v/v) and 100 mg/kg body weight of vitamin C

Group G: 6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of vitamin C

Group H: 6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of vitamin C

Procedure for blood collection and determination of blood CO_2 levels

Blood was collected from the retro-orbital sinus, six hours after the admiration of the last treatment, after blood collection, the sample was centrifuged at 1200rpm for fifteen minutes, the supernatant was then analysed for CO_2 level using an automated electrolyte analyser (SFRI ISE6000-France)

Procedure for determination of antioxidant enzymes activity and lipid peroxidation level of the lungs

The lungs tissue to be accessed for oxidative stress and level of lipid peroxidation, was homogenized in phosphate buffer. Glutathione Reductace (GSH) activity of the lungs was determined using the method described by Sedlak and Lindsay (1968). Catalase (CAT) activities of the lungs was determined by the method described by Sinha (1972), while Superoxide Dismutase (SOD) activity of the lungs was determined accoding to the method of Sun and Zigman (1978). Level of lipid peroxidation (malondiadehyde, MDA) was measure accoding to the methods of Buege and Aust (1978).

Histological examination

After harvesting the lung tissues, it was fixed in a 10% neutral buffered formalin, it was later embedded in paraffin and 5 µm thick sections were prepared and stained with heamatoxylin and eosin using standard procedures. The slides were viewed under light microscope (CELESTRON LCD DIGITAL MICROSCOPE, MODEL 44348) and photomicrographs were taken (200×)

Statistical analysis

All analysis was done using SPSS (version 16) and Microsoft Excel (2019) using and student T-test. Data were expressed as Mean± SEM with $p<0.05$ considered statistically significant. in the results section;

[a]-Values were significant when compared to Group A,
[b]-Values were significant when compared to Group B,
[c]-Values were significant when compared to Group C,
[d]-Values were significant when compared to Group D,
[e]-Values were significant when compared to Group E,
[f]-Values were significant when compared to Group F,
[g]-Values were significant when compared to Group G.

RESULTS AND DISCUSSION

Protective effect of vitamin C against alcohol induced pathological changes on the CO_2 level in adult male Wistar rats

Figure 1 represents the protective activity of vitamin C against alcohol-induced pathological changes in the CO_2 concentration in male Wistar rats. The CO_2 levels in test groups D and E were significantly lower than those in the control group, while the levels in test Group B were significantly higher when compared to other groups, including the control group. When compared to test groups C, D, E, F, G, and H, there was a significant decrease in the carbon dioxide level of rats administered with 6000 mg/kg of alcohol. In rats administered 100 mg/kg of vitamin C, there was a significant decrease in the plasma carbon dioxide level when compared to test groups D, E, and H. There was a significant increase in the plasma carbon dioxide level of rats administered with 300 mg/kg of vitamin C when compared to test Group F.

Metabolic acidosis is a condition that arises when the body generates too much acid or when the organs that are responsible for the elimination of acidic radicals cannot remove or neutralise enough acid from the body. Acute or chronic intoxication from acid-producing substances, including alcohol, chemicals, and some acidiosis-forming medications, is mainly responsible for metabolic acidiosis. The human body has multifactorial physiological

mechanisms to neutralise these acids and remove them from the body via Carbon Dioxide (CO_2) and the Bicarbonate Ion (HCO_3^-), thereby regulating the pH of the blood (Melamed and Melamed 2014). When the CO_2 levels in the blood are high, the concentration of the H^+ ions in the body increases, which will lead to the lowering of the blood pH, causing the pathological state of metabolic acidosis. In our study, the groups administered with alcohol only showed an increase in CO_2 in the blood; this indicated alcohol-induced hypercapnia, which corresponds with the previous study of Sabino et al. (2014). Vitamin C, on the other hand, has a protective effect on the lungs by decreasing systemic oxidative stress, increasing nitric oxide bioavailability, and also restoring vascular endothelial function (Hartmann et al. 2015; Carr and Maggini 2020). The protective effect of vitamin C can be seen across the coadministration Group And in the vitamin C group only.

Protective effect of vitamin C against alcohol induced oxidative stress in the lungs tissue of male Wistar rats

The graphs in Figures 2-5 show the antioxidant effect of vitamin C against alcohol-induced oxidative stress and an increase in lipid peroxidation in the lung tissues of male Wistar rats. The results showed that the consumption of alcohol led to a significant decrease in the antioxidant enzyme activity of the lungs (Figures 2-4) and an increase in the level of lipid peroxidation (Figure 5). In contrast, groups treated with vitamin C showed a significant increase in antioxidant enzyme activity and a decrease in lipid peroxidation levels. Group H, which received the highest dose of vitamin C after alcohol treatment, showed the most significant improvement in antioxidant enzyme activity and levels of lipid peroxidation in comparison to other groups. Oxidants are generated endogenously by metabolic reactions or derived from exogenous sources, and biological systems are constantly exposed to them (Bhattacharya 2015). The human lungs are exposed to a high level of oxygen, which makes them highly susceptible to oxidative injury mediated by free radicals, together with their large surface area and blood supply (Lodovici and Bigagli 2011). The high level of oxidant and the low level of antioxidant will lead to the oxidation of DNA molecules, proteins, and lipids in the cell, as well as inducing different cellular responses through the generation of secondary metabolic free radicals (Di Rosanna and Salvatore 2012). Recent studies have shown that oxidative stress plays an important role in the generation and development of various respiratory pathologies, including asthma, COPD, acute lung injury, lung cancer, and pulmonary fibrosis (Di Rosanna and Salvatore 2012; Liu and Chen 2017). The consumption of alcohol increases oxidative stress through different mechanisms, including the generation of superoxide anion and the production of free radicals at the microsomal level (Jing et al. 2012). According to Table 1 below, the administration of alcohol leads to a decrease in the level of CAT, SOD, and GSH enzyme activity in the lungs. This reduction is also in line with the study of Macdonald et al. (2010). The consumption of alcohol not only activates free radicals but also alters the level of enzymatic and non-enzymatic endogenous antioxidant systems; this will result in oxidative stress with a cascade of effects leading to pathological changes in both the functional and structural integrity of the cell and its organelle membrane (DeLeve et al. 1996), which can also be seen in plate 1b below. The disruption seen in plate 1b below can also be linked to the high level of lipid peroxidation, which will affect the lipid layer of the cell membrane and can only be seen in the alcohol groups. This shows that the consumption of alcohol leads to the disruption of the antioxidant defense system.

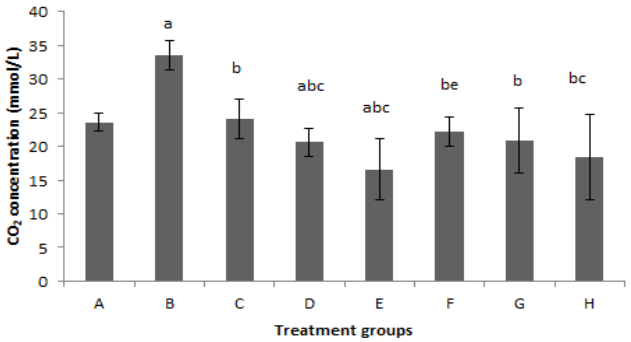

Figure 1. Protective effect of vitamin C against alcohol induced pathological changes on the CO_2 level in adult male Wistar rats. Note: Each bar is an expression of mean ± SEM. ($P < 0.05$). A. Values were significant when compared to Group A, B. Values were significant when compared to Group B, C. Values were significant when compared to Group C, D. Values were significant when compared to Group D, E. Values were significant when compared to Group E, F. Values were significant when compared to Group F, G. Values were significant when compared to Group G

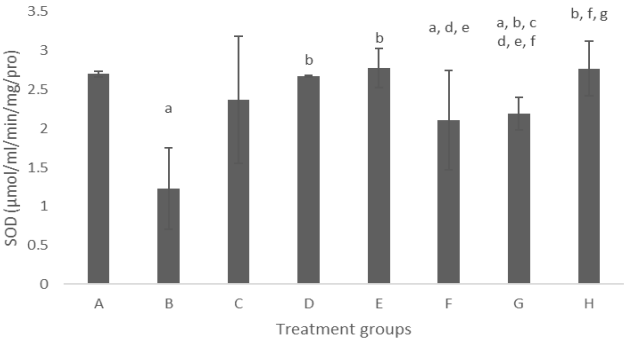

Figure 2. Protective effect of vitamin C against alcohol induced pathological changes on the SOD activity in the lungs tissue of male Wistar rats. Note: Each bar is an expression of mean ± SEM. ($P < 0.05$). A. Values were significant when compared to Group A, B. Values were significant when compared to Group B, C. Values were significant when compared to Group C, D. Values were significant when compared to Group D, E. Values were significant when compared to Group E, F. Values were significant when compared to Group F, G. Values were significant when compared to Group G

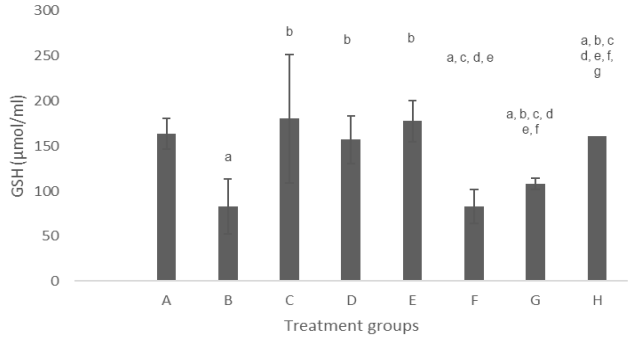

Figure 3. Protective effect of vitamin C against alcohol induced pathological changes on the GSH activity in the lungs tissue of male Wistar rats. Note: Each bar is an expression of mean ± SEM. (P <0.05). A. Values were significant when compared to Group A, B. Values were significant when compared to Group B, C. Values were significant when compared to Group C, D. Values were significant when compared to Group D, E. Values were significant when compared to Group E, F. Values were significant when compared to Group F, G. Values were significant when compared to Group G

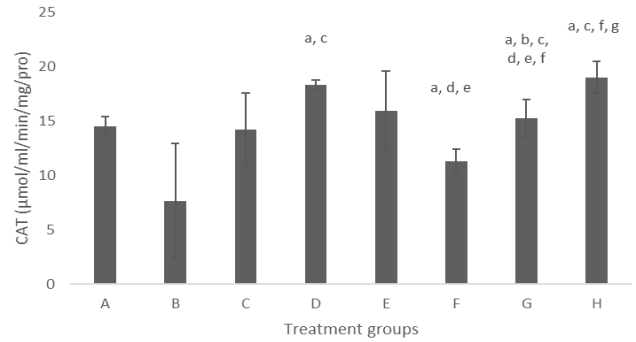

Figure 4. Protective effect of vitamin C against alcohol induced pathological changes on the CAT activity in the lungs tissue of male Wistar rats. Note: Each bar is an expression of mean ± SEM. (P <0.05). A. Values were significant when compared to Group A, B. Values were significant when compared to Group B, C. Values were significant when compared to Group C, D. Values were significant when compared to Group D, E. Values were significant when compared to Group E, F. Values were significant when compared to Group F, G. Values were significant when compared to Group G

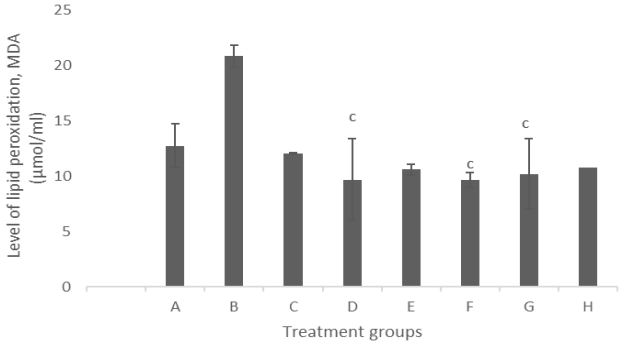

Figure 5. Protective effect of vitamin C against alcohol induced pathological changes on the level of lipid peroxidation (MDA) in the lungs tissue of male Wistar rats. Note: Each bar is an expression of mean ± SEM. (P <0.05). A. Values were significant when compared to Group A, B. Values were significant when compared to Group B, C. Values were significant when compared to Group C, D. Values were significant when compared to Group D, E. Values were significant when compared to Group E, F. Values were significant when compared to Group F, G. Values were significant when compared to Group G

A therapeutic approach to alcohol-induced oxidative stress may involve the use of exogenous and endogenous antioxidant entities (Xu et al. 2020). Recent research involving the use of antioxidants such as vitamin C and E has shown that these vitamins to an extent exert antioxidative and anti-inflammatory activity on lung tissue (Domej et al. 2014; Adewoyin et al. 2017; Liu et al. 2018). The results of our study showed that the administration of vitamin C has both antioxidant and inflammatory effects on alcohol-induced lung toxicity.

Protective effect of vitamin C against alcohol induced tissue damage on the histo- architecture of the lungs in male Wistar rats

The toxic effect of alcohol on the respiratory system is underappreciated. This is due to the fact that there are no pathological changes noticed at first until there is a secondary insult. Chronic ethanol ingestions impact all aspects of the alveolar epithelium. This is due to the fact that the alveoli are rich in blood supply, alcohol is absorbed and distributed in an unaltered state, meaning it is not bound to any protein or transported via any specific transport mechanisms, and the lungs are the most vulnerable organs after the ingestion of alcohol (Downs et al. 2013). The study of Brown and Brown (2012) revealed that chronic consumption of alcohol will lead to a pathological impairment in alveolar macrophage function that will further degenerate into decreased phagocytosis and increased reactive oxygen species production, as seen in Table 1.

In Figure 6.B, there was a dilated alveolar sac, which indicated that the walls of the airspace below the terminal bronchioles were damaged. When the alveoli are dilated, the structure of the alveoli is destroyed, the elasticity is reduced and lost, leading to air stagnation, which will lead to the impairment of the gas exchange function of the alveoli. The dilation of the alveoli will also complement the alcohol-induced bronchioles disorientation. These pathological changes are caused by an Alpha-1 Antitrypsin (AAT) protein deficiency. Previous studies have shown that the consumption of alcohol is responsible for the deficiency of AAT proteins; the AAT proteins have a protective effect on the elasticity of the alveoli, and their deficiency will lead to dilation of the alveoli (Senn et al. 2008; Hoth et al. 2012). Since alcohol also plays an important role in the development of oxidative stress (Kahraman et al. 2012), according to the study of Dasi et al. (2013), oxidative stress also leads to a deficiency of the AAT protein.

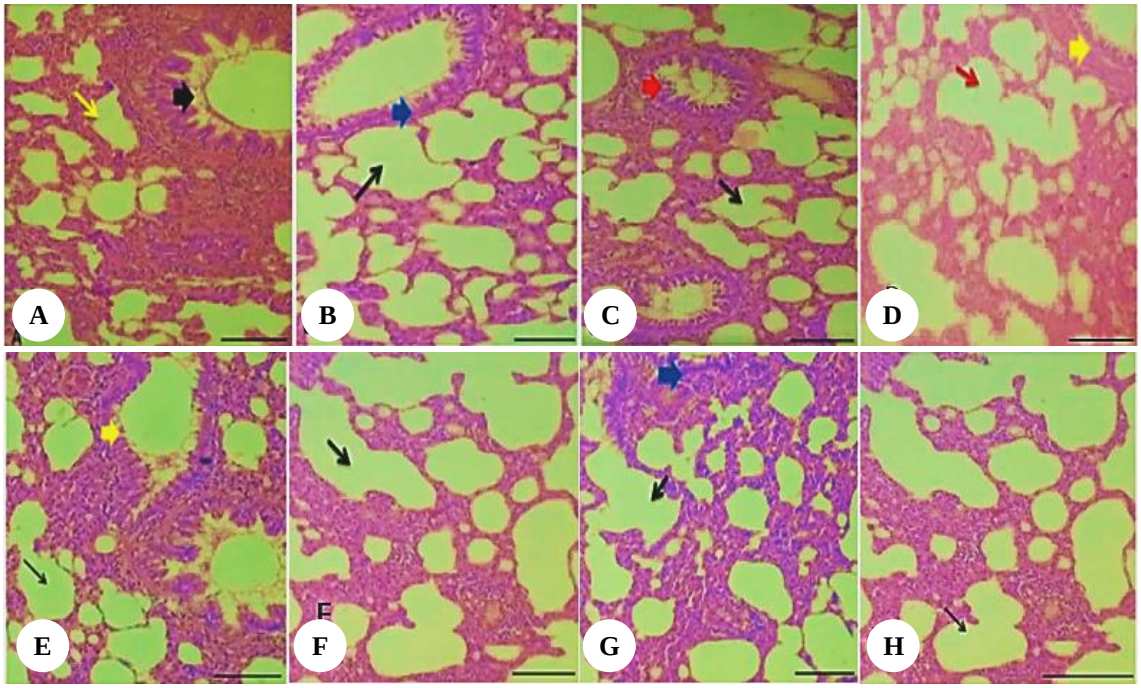

Figure 6. Protective effect of vitamin C against alcohol induced tissue damage on the histo- architecture of the lungs in male Wistar rats H/E X200. Scale Bar =120µm

The toxic effect of free radicals can be canceled out by antioxidants, which may be dietary, endogenous, enzymatic, or non-enzymatic, through various mechanisms, such as electron donation, catalytic removal, binding radicals, and gene expression regulation. Together, antioxidants constitute an integrated defense against ROS and the development of oxidative stress (Janciauskiene 2020). Previous studies have shown that vitamin C and other classes of antioxidants have a protective effect on lung function (Grievink et al. 1998; Ares et al. 2013). In our study, the administration of vitamin C caused regenerative changes in the histo-architecture of the lungs. This is due to the fact that vitamin C works by increasing Nrf2 expression and protein levels and, concomitantly, increasing the activity of antioxidant defense.

Figure 6 shows the protective effect of vitamin C against alcohol-induced lung toxicity. In the control group's lung tissue, there was a well-differentiated bronchiole (black thick arrow), alveolar sac (yellow thin arrow), and alveolar septa. In the group treated with 6000 mg/kg of alcohol, there was a dilated alveolar sac (black thin arrow) and disorientation of the bronchiole (blue thick arrow) with loss of function. In test Group C, there were no pathological histomorphological changes; the bronchiole (red thick arrow) and the alveoli sac (black thin arrow) are well organized. Test Group D has no pathological histomorphological changes; the bronchiole (yellow thick arrow) and the alveoli sac (red thin arrow) are well organized. Also, test Group E showed no pathological histomorphological changes; the bronchiole (yellow thick arrow) and the alveoli sac (black thin arrow) are well organized. In Group F, there was well-regenerated and improved lung tissue with a slight dilation of the alveoli sack. Test Group G showed well-regenerated and improved lung tissue with slight dilation of the alveoli sac (black thin arrow), while test group H showed well-regenerated and improved lung tissue with slight dilation of the alveoli sac (black thin arrow).

In conclusion, the consumption of alcohol has been shown to cause an increase in oxidative stress, which will lead to a decrease in antioxidant enzyme activity. In our study, vitamin C showed a protective effect against alcohol-induced toxicity in the lungs of adult male Wistar rats.

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