Original Article

Protective Effect of Commercial Grade Vitamin C against Alcohol-induced Testicular Damage in Male Wistar Rats

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Abstract

Background: Alcohol consumption has a negative effect on male fertility, but Vitamin C may be able to alleviate this effect. **Aims:** In this study, the protective effect of Vitamin C against alcohol-induced testicular damage in adult male Wistar rats was evaluated. **Settings and Design:** This study was conducted in a University setting. Following a 14-day acclimatisation period, forty adult male Wistar rats were randomly divided into eight groups of five rats. The control group received only food and water, test group B received alcohol only, test group C to E received different doses of Vitamin C, test group F to G received different doses of Vitamin C and alcohol. **Materials and Methods:** After a 21-day treatment period, the testis were harvested and analysed for sperm parameters, antioxidant enzyme activity, level of lipid peroxidation and histopathological changes. **Statistical Analysis Used:** All analyses was performed using SPSS (version 16) and Microsoft Excel (2019) using Student's *t*-test. **Results:** The results showed that in groups administered with alcohol only, there was a decrease in sperm count. Sperm motility, morphology, viability and antioxidant enzyme activity, but increase in the level of lipid peroxidation. In groups treated with Vitamin C and alcohol, there was improvement in the sperm parameters, antioxidant enzymes activity and a decrease and decrease in lipid peroxidation. Furthermore, in the histology of the testis, regenerative changes were seen. **Conclusion:** The chronic consumption of alcohol can have a deleterious effect on the testis, but commercial-grade Vitamin C can reverse these effects.

Keywords: *Alcohol, antioxidant, lipid peroxidation, male fertility, Vitamin C*

Introduction

Vitamin C, is often referred to as ascorbic acid (AA); it is the body's most efficient concentration-dependent water-soluble antioxidant.[1] AA aids in enzyme activation, oxidative stress reduction and immune system enhancement. Research has shown that it protects against respiratory infections, reduces the risk of cardiovascular diseases and may prevent the development of malignancies.[2,3] Mangoes, pineapples and berries are just a few examples of fresh fruit and vegetable that naturally contain AA. Most plants and animals also produce it for their own needs.[4,5] Humans, unlike most animals lack the gluconolactone oxidase enzymes, so therefore are unable to synthesize AA endogenously; hence, they get it from fruits, vegetables and supplements.[6] Due to their antioxidant qualities, AA and its derivatives are frequently used as preservatives in the food business in the food business. It is necessary for the body's fundamental physiological processes and has a wide range of health advantages, including anti-carcinogenic, anti-atherogenic and antioxidant qualities. It helps in

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the hydroxylation of glycine, proline, lysine, carnitine and catecholamines, as well as the synthesis and metabolism of tyrosine, folic acid and tryptophan,[7] AA decreases blood cholesterol levels by promoting the conversion of cholesterol to bile acids and enhancing iron absorption in the intestine through the conversion of ferric to ferrous. As an antioxidant, it protects the body from the harmful effects of free radicals, pollutions and toxins.[8]

Ethanol is commonly referred to as ethyl alcohol, it is predominantly metabolised in the liver, where it produces toxic by-products, including acetaldehyde and acetate, which will lead to the development of oxidative stress and the production of free radicals. Furthermore, ethanol can interact with lipids to create phosphatidyl-ethanol and fatty acid ethyl esters, both of which can disrupt regular cellular functions.[9]

The male reproductive system consists of external tissues like the scrotum and penis and internal organs such as the testis, epididymis, vas deferens and prostate. The development, storage and ejaculation of sperm for fertilisation as well as the synthesis of essential androgens for male growth, are all facilitated by these tissues' extensive vascularisation with an abundance of glands and ducts. The most crucial male androgen is the testosterone, which is produced by the Leydig cell of the testis.[10] In both experimental and clinical studies, drinking alcohol has been connected with pathological alteration in testosterone release and spermatogenesis. In fact, considerable morphological alterations in sperm, such as head breaking, middle distension and tail curling, are well-known effect of alcohol consumption. In addition, azoospermia is caused by faulty spermatids that are frequently found in the seminiferous tubules of alcoholics. The functioning of the hypothalamic pituitary testicular axis may be altered as a result of the impacts, as well as a direct influence on the testis and other male reproductive accessory glands; alcohol consumption is associated with pathological changes in the physiological function of the male reproductive system and sperm parameters.[11,12]

Despite alcohol's social acceptance, it detrimental effects on male reproductive function have been well documented. Numerous studies over the last few decades have found a variety of harmful effect of alcohol consumption on male fertility.[13] Conversely, Vitamin C is a safe and potent antioxidant,[1] that has been shown the have therapeutic effects on male reproductive function.[14,15] This study aimed to evaluate the protective effect of Vitamin C against alcohol-induced testicular damage in adult male Wistar rats.

Subjects and Methods

Animal care and grouping

For this experiment, 40 adults healthy Wistar male rats weighing 150 g to 250 g were utilised. The rats were housed in wire and plastic cages in the Animal House of the Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria. The rats were given 2 weeks to acclimatize; they were fed with a standard pellet diet and given unrestricted access to water. The national research council[16] internationally recognised 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, 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 therapy for 21 days.

- 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 determination of antioxidant enzymes activity of the testis

The testis tissue to be accessed for oxidative stress and level of lipid peroxidation was homogenised in phosphate buffer. Glutathione reductase (GSH) activity of the testis was determined using the method described by Sedlak and Lindsay,[17] level of lipid peroxidation (malondialdehyde [MDA]) was determined using the method described by Buege and Aust.[18] Catalase (CAT activities of the testis was determined by the method described by Sinha,[19] while superoxide dismutase (SOD) activity of the testis was determined by the method of Sun and Zigman.[20]

Procedure for evaluation of sperm parameters

Sperm was collected from the epididymis using the diffusion method described by Seed *et al.*,[21] The determination and classification of sperm morphology

and sperm count and motility were done according to the method of Saalu *et al*.[22]

Histological examination

After harvesting the testis tissue 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 haematoxylin and eosin using standard procedures. The slides were viewed under a light microscope and photomicrographs were taken (×200).

Statistical analysis

All analysis was performed using SPSS (version 16) IBM, New York, United State and Microsoft Excel (2019) using one-way Student's *t*-test. Data were expressed as mean ± standard error of the mean with $P < 0.05$ considered statistically significant.

Results

Effect of Vitamin C changes caused by alcohol on the weight of the testis

The graph in Figure 1 illustrates the protective activity of Vitamin C against alcohol-induced pathological changes in the relative weight of the testis in male Wistar rats. Group B had much lighter testicles than Group A. The testis weight of Group C, which received 100 mg/kg of Vitamin C, did not change significantly from that of Group A. Group D had a testicular weight that was significantly greater than those of Group B. The testis weight of Group E was much higher than that of Group B and Group D. The testis weights of Groups F, G and H were significantly greater than those of the B group. Group H, which received the highest dose of Vitamin C (300 mg/kg body weight), had the highest testis weight. Overall, the results suggest that alcohol administration decreases testis weight in rats, while Vitamin C supplementation can partially mitigate this effect. The highest dose of Vitamin C (300 mg/kg body weight) was the most effective in preventing the decrease in testis weight caused by alcohol.

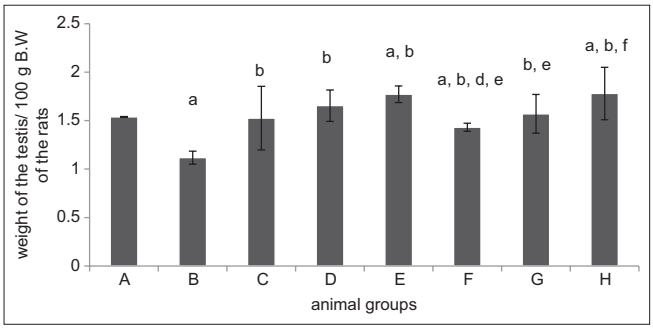

Figure 1: Effect of the concurrent administration of alcohol and Vitamin C on the weight of the testis in adult male Wistar rats. Each bar is an expression of mean ± SEM. ($P < 0.05$). a-Values were significant when compared to group A, b- values were significant when compared to group B, c- values were significant when compared to group C, d- values were significant when compared to D, e- values were significant when compared to E, f- values were significant when compared to F. SEM = Standard error of the mean

Effect of alcohol and Vitamin C interaction on the antioxidant enzymes activity and level of lipid peroxidation in the testis

Table 1 provides measurements of GSH (glutathione), SOD, CAT and MDA in each group. The results indicate that alcohol administration significantly decreased the activity of antioxidant enzymes and increased lipid peroxidation in the testis, while Vitamin C treatment was found to counteract the negative effects of alcohol on these parameters. Increasing doses of Vitamin C led to a corresponding increase in antioxidant activity and a decrease in lipid peroxidation, indicating a dose-dependent relationship.

Effect of the concurrent administration of alcohol and Vitamin C on selected sperm parameters adult male Wistar rats

Table 2 shows the effect of concurrent administration of alcohol and Vitamin C on selected sperm parameters in adult male Wistar rats. Vitamin C supplementation at all doses tested (Groups C, D, E and H) led to a significant improvement in sperm count when compared to the alcohol-only group (Group B). Higher doses of Vitamin C (Groups D, E and H) also significantly improved normal morphology and sperm motility compared to the alcohol-only group. Interestingly, the combination of high-dose alcohol and low-dose Vitamin C (Group F) did not show any significant improvement in sperm parameters compared to the alcohol-only group. However, when higher doses of Vitamin C were combined with alcohol (Groups G and H), there was a significant improvement in all measured sperm parameters compared to the alcohol-only group. Overall, the results suggest that Vitamin C supplementation can improve sperm count, normal morphology and sperm motility in rats exposed to alcohol, and the dose of Vitamin C appears to be an essential factor in determining its efficacy.

Effect of the concurrent administration of alcohol and Vitamin C on the histoarchitecture of the testis of adult male Wistar rats

Histomorphology observations revealed that the control group's testicular tissue displayed normal and well-defined spermatogonia cells (black circle), Leydig cells in the interstitial layer (IL) (thin black arrow), sertoli cells (thin red arrow) and a lumen (L) containing many late spermatids. In rats that received 6000 mg/kg of alcohol, testicular atrophy and inconsistencies in the seminiferous tubules' diameter (thick red arrow), Leydig cells in the IL, spermatogonia cells (thin black arrow)

and sertoli cells (yellow arrow) were observed. Groups treated with 100, 200 and 300 mg/kg of commercial grade Vitamin C showed mild testicular atrophy and inconsistencies, along with expanded seminiferous tubules (thick red arrow) and well-defined spermatogonia cells (thin black arrow), sertoli cells (thin red arrow) and Leydig cells in the IL. In the groups treated with both 6000 mg/kg of alcohol and 100 mg/kg or 200 mg/kg of commercial grade Vitamin C, mild testicular atrophy, expanded seminiferous tubules (thick yellow arrow), reduced spermatocytes, well-defined spermatogonia cells (red circle) and Leydig cells in the IL were observed. In the testicular tissue of rats treated with both 6000 mg/kg and 300 mg/kg of alcohol and commercial Vitamin C, the seminiferous tubule (thick red arrow), spermatogonia cells (yellow circle) and Leydig cells in the IL were well-defined, indicating normal testicular morphology.

Discussion

Alcohol-induced oxidative stress is associated with ethanol metabolism, which includes alcohol dehydrogenase, the microsomal ethanol oxidation system and CAT.[23] The metabolism of ethanol by alcohol dehydrogenase leads to the formation of acetaldehyde, which produces free radicals that affect the antioxidant defence system.[24-26] Reduced glutathione (GSH) is a crucial antioxidant that reduces the toxicity of ethanol

Table 1: Effect of the concurrent administration of alcohol and Vitamin C on the antioxidant enzyme activity and level of lipid peroxidation in the heart of adult male Wistar rats

Group	Treatment	GSH (µmol/mL)	SOD (µmol/mL/min/mg/pro)	CAT (µmol/mL/min/mg/pro)	MDA (µmol/mL)
A	Distilled water only	208.40±17.37	1.60±0.11	7.79±1.76	15.79±5.38
B	6000 mg/kg body weight of alcohol (30% v/v)	137.52±32.39^{A}	0.79±0.40^{A}	6.53±2.63	30.95±9.69^{A}
C	100 mg/kg of body weight of Vitamin C	230.58±57.65^{B}	1.92±0.10^{B}	12.29±1.27	10.72±1.31^{A,B}
D	200 mg/kg body weight of Vitamin C	236.48±26.71^{A,B}	2.31±0.29^{A,B,C}	14.61±2.40^{A,B,C}	10.71±8.16^{B}
E	300 mg/kg body weight of Vitamin C	254.65±51.51^{A,B}	2.43±0.63	16.64±3.58^{A,B,C}	8.64±3.24^{A,B}
F	6000 mg/kg body weight of alcohol and 100 mg/kg body weight of Vitamin C	163.22±6.42^{A,C,D,E}	1.76±0.15^{A,B,C,D}	8.90±1.40^{D,E}	14.89±3.74^{B,C,E}
G	6000 mg/kg body weight of alcohol and 200 mg/kg body weight of Vitamin C	175.69±53.02^{D,E}	1.8±0.17^{A,B,C,D}	10.1±0.16^{A,B,C,D,F}	10.91±4.08^{B}
H	6000 mg/kg body weight of alcohol and 300 mg/kg body weight of vitamin	248.10±10.79^{A,B,F,G}	2.25±0.79^{B}	12.45±3.70^{A,B,E,F}	11.91±7.25^{B}

^{A}Significant when compared to group A, ^{B}Significant when compared to B, ^{C}Significant when compared to C, ^{D}Significant when compared to D, ^{E}Significant when compared to E, ^{F}Significant when compared to F, ^{G}Significant when compared to G. Each value is an expression of mean±SEM (P<0.05). SEM=Standard error of the mean, GSH=Glutathione reductace, SOD=Superoxide dismutase, CAT=Catalase, MDA=Malondialdehyde

Table 2: Effect of the concurrent administration of alcohol and Vitamin C on selected sperm parameters adult male Wistar rats

Group	Treatment	Normal morphology (%)	Sperm motile (%)	Sperm count (×10^{6} m/L)
A	Distilled water only	70±0	75±7.07	70.24±0.07
B	6000 mg/kg body weight of alcohol (30% v/v)	26.00±0.45^{A}	35±21.21^{A}	26.99±0.24
C	100 mg/kg of body weight of Vitamin C	60.00±0.58^{A}	60±0^{A}	73.44±0.91^{A}
D	200 mg/kg body weight of Vitamin C	75.21±0.89^{A,B}	70.56±11.34^{A}	81.00±0.00^{A}
E	300 mg/kg body weight of Vitamin C	80.66±2.44	82±2.44^{A}	81.00±0.71^{A}
F	6000 mg/kg body weight of alcohol and 100 mg/kg body weight of Vitamin C	41.98±9.333	55±6.07^{A,B}	37.45±0.37^{A,B}
G	6000 mg/kg body weight of alcohol and 200 mg/kg body weight of Vitamin C	50.00±0.00^{A,B,D}	65±7.07^{A,D}	71.90±0.44^{B}
H	6000 mg/kg body weight of alcohol and 300 mg/kg body weight of vitamin	67.78±19.44^{A,B,C,E}	67.5±3.53^{A,B,E}	73.24±0.56^{A,B}

^{A}Significant when compared to group A, ^{B}Significant when compared to B, ^{C}Significant when compared to C, ^{D}Significant when compared to D, ^{E}Significant when compared to E, ^{F}Significant when compared to F, ^{G}Significant when compared to G. Each value is an expression of mean±SEM (P<0.05). SEM=Standard error of the mean

and other toxic products in the body.[27] Cytochrome 2E1-expressing cells lose viability due to GSH depletion, which damages the mitochondrial membrane potential. Alcohol has a number of deleterious effects on several organs, including oxidative stress brought on by cytochrome 2E1 expressing cells, mitochondrial damage, stimulation of stellate cells and GSH homeostasis. The consumption of alcohol lowers the free SH-group concentration and hydrogen ability, which causes the generation of oxidants, inhibition of the mitochondrial glutathione transporter and depletion of GSH as seen in the groups which received alcohol alone.[28] The upsurge in the level of lipid peroxidation is also correlated with the reduction in GSH enzyme activity. Elevated MDA levels are associated with lower GHS levels and an increase in free radicals, this is because MDA and GSH have a string and reciprocal interaction.[29]

SOD enzymes are essential for controlling cellular ROS levels and the reduction in SOD activity makes cells more susceptible to oxidative stress and the onset of metabolic diseases.[30] Alcohol administration to rats in the study resulted in decreased SOD activity, demonstrating the connection between alcohol consumption and the development of oxidative stress. Previous study has shown that the continuous consumption of alcohol may initially increase SOD expression in healthy controls, preventing ethanol-induced oxidative damage brought on by the body's immunological reaction to toxins. The adaptive response of SOD reduces and the elevated SOD gradually decreases with repeated alcohol consumption increasing the toxicity of protracted ethanol exposure.[28,31]

CAT is a critical antioxidant that converts excessive oxygen that may evolve into hydroxyl radicals into hydrogen peroxide and then water and oxygen, thereby preventing DNA mutation and the development of diseases.[32] The consumption of alcohol is often linked with the development of diseases and alcohol-induced oxidative stress.[33] The body increased production of the enzymes needed to carry electrons during metabolism, nicotinamide adenine dinucleotide, brought on by the metabolism of ethanol, causes an increase in respiration and oxygen consumption, which in turn causes an increase in oxidative stress and a decrease in CAT activity,[34] as shown in Table 1 above.

According to previous research, there is a relationship between alcohol consumption and testicular damage.[35-37] According to Dosumu *et al*.,[35] the consumption of alcohol affects the mitochondrial ability to carry out protein synthesis due to alcohol-induced alteration in the mitochondria ribosomes. The alteration can lead to enzyme deactivation, apoptotic and necrotic cell death, which is a significant factor in alcohol-induced testicular damage. According to our findings rats' administered with alcohol showed abnormalities and degradation of the IL, lumen and seminiferous tubules, as seen in Plate 1 above. The consumption of alcohol increases the formation of free radicals, which results in improper activation of the mitochondrial permeability transition and pro-apoptotic pathways,[38] contributing to the damage seen in the testis histology. According to Zhu *et al*.,[39] the testicular atrophy may be caused by ethanol-induced augmentation of the germ cells apoptosis, necrosis

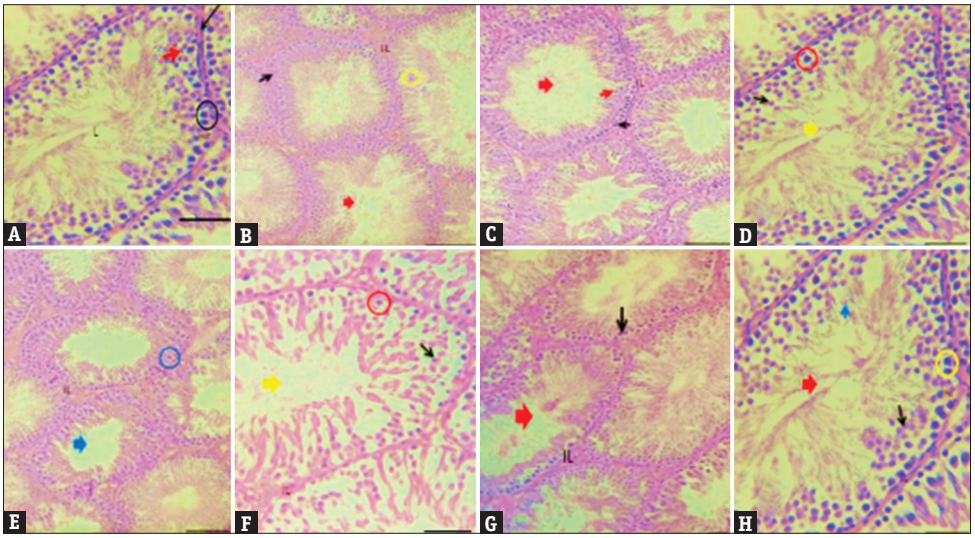

Plate 1: Effect of the concurrent administration of alcohol and commercial grade Vitamin C on the liver histology H/E × 200. Scale Bar = 60 µm. A; Distilled water only, B; 6000 mg/kg body weight of alcohol (30% v/v), C; 100 mg/kg body weight of Vitamin C, D; 200 mg/kg body weight of Vitamin C, E; 300 mg/kg body weight of Vitamin C, F; 6000 mg/kg body weight of alcohol (30% v/v) and 100 mg/Kg body weight of Vitamin C after 2 h, G; 6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of Vitamin C after 2 h, H; 6000 mg/kg body weight body weight of Vitamin C after 2 h

and reduction of cell growth. Alcohol consumption may have a negative impact on sperm parameters, which will ultimately have an effect on male fertility. Alcohol toxicity on sperm parameters is a complex, multifaceted process that includes oxidative stress, testicular injury and other factors already mentioned above. The consumption of alcohol might prevent the body from absorbing nutrients such as zinc, folate and Vitamin C that are necessary for sperm development and function. For instance, folate is responsible for the synthesis and integrity of DNA, whereas zinc is important for spermatogenesis. Low sperm count and aberrant morphology are caused by certain nutritional deficiencies.[40,41]

In an invitro cell-free system Vitamin C can also acts as a chain-breaking antioxidant of lipid peroxidation, preventing oxidative stress and organ damage,[42] the therapeutic activities of Vitamin C are shown in Table 1, as it reduces oxidative stress in groups F, G and H. Vitamin C is important when it comes to male fertility, as an antioxidant it shields the sperm from oxidative stress and DNA deterioration brought on by free radicals, also AA is needed for the production of collagen, which is the structural element of the testis,[43] this is also seen the histoarchtecture of the testis as in group treated with Vitamin C there were therapeutic changes seen. In addition to its antioxidant properties, AA can improve sperm count, motility and morphology. It has been shown to increase the number of motile sperm, decrease the number of immotile sperm and improve the morphology of sperm cells. AA can also enhance the production of seminal fluid, which provides nutrients and support for the sperm. Furthermore, AA can enhance the absorption of other nutrients necessary for male fertility, such as zinc and folate.[44]

Conclusions

The results showed that alcohol administration caused an increase in lipid peroxidation levels, low antioxidant enzyme activity, decrease in sperm parameters and testicular atrophy. However, in groups treated with alcohol and Vitamin C, there was an increase in antioxidant enzyme activity, a reduction in lipid peroxidation levels and regenerative changes in the histo-architecture of the testis. The study concludes that Vitamin C can reverse the deleterious effects of alcohol on the testes.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Data availability statement

The data used in this study are available with the corresponding author who is willing to share it upon reasonable request.

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