Original Research

Ameliorative Activity of Vitamin C against Alcohol Induced Cardio-toxicity in Adult Male Wistar Rats

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ABSTRACT

Background: Alcohol and it metabolites causes cardiomyopathy, which is one of the main form of alcohol induced heart damage. The aim of the present study was to investigate the protective effect of vitamin C against alcohol-induced heart toxicity and selected indices. **Material and Methods:** Forty healthy male Wistar rats were used in this experiment, which lasted 21 days. The rats were divided into eight groups, with five rats in each group. Group A received only distilled water, Group B received 6000 mg/kg of alcohol, and Groups C to H received different doses of vitamin C and alcohol in varying combinations. Blood samples were collected and analyzed for levels of sodium, potassium, and chloride ions. The hearts were also analyzed for antioxidant activities and histo-pathological changes. **Results:** The findings indicated that alcohol administration caused a decrease in blood electrolyte levels compared to the control group, while treatment with vitamin C and co-administration of vitamin C and alcohol improved blood electrolyte levels. The antioxidant enzymes activity of the heart improved in the vitamin C and co-administration groups, as evidenced by increased GSH, SOD, and CAT activity and decreased MDA levels when compared to the alcohol-only group. **Conclusion:** Therefore, this study suggests that commercial grade vitamin C at doses of 100 mg/kg, 200 mg/kg, and 300 mg/kg can protect the heart against alcohol-induced toxicity. However, further research is necessary to examine the anti-inflammatory effect of commercial grade vitamin C.

Highlights:

1. Ameliorative effects of vitamin C against alcohol in this research are very interesting to be observed.
2. Vitamin C may have some beneficial effects on heart function by acting as an antioxidant.

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Introduction

The importance of Vitamin C in many biological reactions cannot be overstated, as it is an essential water-soluble nutrient that cannot be synthesized by humans and therefore it is gotten from external sources e.g., fruits [1]. Vitamin C plays a critical role in preserving essential tissue structure and physiological function by being involved in the biosynthesis of collagen, without which capillary fragility develops [2]. In addition to its role in collagen synthesis, Vitamin C is involved in several roles in cellular metabolism, including oxidation-reduction reactions and serving as an enzyme co-factor. [3]

Alcohol consumption has been shown to compromise oxidative stress and nutritional status of the body [4]. Chronic alcohol abuse can lead to malnourishment due to reduced intake of essential nutrients or impaired absorption of essential elements [5]. The metabolic pathways of ethanol can generate intermediate toxic products, such as acetaldehyde and free radicals, which interfere with the normal metabolism of essential elements, leading to cellular damage through oxidation mechanisms and secondary oxidative stress inflammation [6]. Chronic or acute consumption of alcohol can lead to degeneration in different internal organs and systems of the body [7], which has become a significant medical and social issue in many countries [8]. Ethanol oxidation in the liver produces acetaldehyde via enzymatic activity in cytosol, microsomes, and peroxisomes, and the oxidation of ethanol by alcohol dehydrogenase generates ROS by various organelles that can cause cellular damage until they are removed by the antioxidant defense system. [9-10]

Vitamin C is known to be a potent antioxidant that can donate a hydrogen atom and form a relatively stable ascorbyl free radical [11]. Oxidative stress can significantly affect the antioxidant enzymes CAT, SOD, and GSH, which play critical roles in removing free radicals [12]. Antioxidant compounds can prevent the uncontrolled formation of free radicals or inhibit their reaction with biological sites. The destruction of most free radicals depends on the oxidation of endogenous antioxidants primarily by scavenging and reducing molecules [13]. The objective of this study is to observe the protective effects of vitamin C on alcohol-induced toxicity on the heart and selected indices.

Material 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. The rats were given two weeks to acclimatize; they were feed with a standard pellet diet and given unrestricted access to water. The national research council [14]

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, Olabisi Onabanjo University, Sagamu, Ogun state, Nigeria, with approval number OOU/PHSECR/22/009.

Subject Groups

Eight groups of five rats each were formed randomly from the rat population, and each group received treatments for 21 days.

1. Group A: distilled water only.
2. Group B: 6000 mg/kg body weight of alcohol (30% v/v)
3. Group C: 100 mg/kg body weight of vitamin C
4. Group D: 200 mg/kg body weight of vitamin C
5. Group E: 300 mg/kg body weight of vitamin C
6. Group F: 6000 mg/kg body weight of alcohol (30%v/v) and 100 mg/kg body weight of vitamin C.
7. Group G: 6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of vitamin C.
8. 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 sodium, potassium, and chloride ion levels

Six hours after the administration of the last treatment, blood was collected from the retro-orbital sinus, the rat was restrained, the neck gently scuffed, and the eye bulged. Then, a capillary tube was inserted medially into the eye and blood was allowed to flow by capillary action through the capillary tube into lithium heparin sample bottle. The blood was then centrifuged at 1200rpm for fifteen minutes, the supernatant was then analyzed for sodium, potassium and chloride ion levels using an automated electrolyte analyzer (SFRI ISE6000-France).

Procedure for determination of antioxidant enzymes activity of the heart

The heart tissue to be accessed for oxidative stress and level of lipid peroxidation, was homogenized in phosphate buffer. Glutathione reductase (GSH) activity of the heart was determined using the method described by Sedlak and Lindsay [15], level of lipid peroxidation (malondialdehyde/MDA) was determined using the method described by Buege and Aust [16]. Catalase (CAT activities of the heart was determined by the method described by Sinha [17], while superoxide dismutase (SOD) activity of the heart was determined by the method of Sun and Zigman. [18]

Histological examination

After, harvesting the heart tissue, it was fixed in a 10% neutral buffered formalin, it was later embedded in paraffin and 5μm thick sections were prepared and stained with hematoxylin and eosin using standard procedures. The slides were viewed under light microscope and photomicrographs were taken (200✕).

Statistical analysis

All analysis was done using SPSS (version 16) and Microsoft excel 2019 one-way ANOVA followed by Student's Newman-Keuls post-hoc test. Data were expressed as Mean ± SEM with $p < 0.05$ considered statistically significant.

Result

Table 1. Ameliorative effect of vitamin C against alcohol induced electrolyte imbalance in male Wistar rats

Group	Treatment	Na$^+$ (mmol/L)	K$^+$ (mmol/L)	Cl$^-$ (mmol/L)
A	Distilled water only	135.80 ±2.28	3.88 ±0.13	100.00 ±1.58
B	6000 mg/kg body weight of alcohol (30% v/v)	103.80±14.92[A]	1.50 ±0.35[A]	77.20 ±22.08[A]
C	100 mg/kg body weight of vitamin C	121.20 ±4.97[AB]	3.80 ±0.12[B]	104.00 ±2.92[AB]
D	200 mg/kg body weight of vitamin C	119.20 ±5.72[AB]	4.08 ±0.13[ABC]	99.20 ±4.55[BC]
E	300 mg/kg body weight of vitamin C	114.80 ±4.09[ABC]	4.10 ±0.10[ABC]	95.00 ±9.72[BC]
F	6000 mg/kg body weight of alcohol (30% v/v) and 100 mg/kg body weight of vitamin C after two hours	137.60 ±0.55[BCDE]	3.64 ±0.55[ABCDE]	102.60 ±0.55[AB]
G	6000 mg/kg body weight of alcohol (30% v/v) and 200 mg/kg body weight of vitamin C after two hours	131.40±12.18[BDE]	3.48±0.43[ABDE]	98.80 ±1.92[BCF]
H	6000 mg/kg body weight of alcohol (30% v/v) and 300 mg/kg body weight of vitamin C after two hours	126.00 ±6.52[ABFG]	3.14 ±0.17[ABCDEF]	97.00 ±4.36[BCF]

Note: Sodium (Na$^+$), potassium (K$^+$), chloride (Cl$^-$)

[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

Ameliorative effect of vitamin C against alcohol induced electrolyte imbalance in male Wistar rats

Table 1 shows the protective effect of vitamin C against alcohol induced electrolyte imbalance in male Wistar rats. Group B, which received 6000 mg/kg body weight of alcohol, showed a significant decrease in Na^+, K^+, and Cl^- levels compared to group A, which received distilled water only. Groups C, D, and E, which received increasing doses of vitamin C, showed no significant changes in Na^+ levels compared to group A, but K^+ and Cl^- levels increased with higher doses of vitamin C. Group F, which received alcohol and 100 mg/kg body weight of vitamin C after two hours, showed a significant increase in Na^+ and Cl^- levels compared to group B. Groups G and H, which received higher doses of vitamin C and alcohol, showed no significant changes in Na^+ levels compared to group B, but K^+ levels increased with higher doses of vitamin C. Group H showed a significant increase in Cl^- levels compared to group G. Overall, vitamin C supplementation appeared to have a positive effect on K^+ and Cl^- levels, and may partially mitigate the negative effects of alcohol on electrolyte balance.

Ameliorative effect of vitamin C against alcohol induced weight changes in male Wistar rats

The graph shows the ameliorative effect of vitamin C against alcohol induced weight changes in male Wistar rats. The y-axis shows the mean weight of the heart (g), and the x-axis shows the different treatment groups. Group A received distilled water only, Group B received 6000mg/kg body weight of alcohol, Group C received 100mg/kg body weight of vitamin C, Group D received 200mg/kg body weight of vitamin C, Group E received 300mg/kg body weight of vitamin C, Group F received 6000mg/kg body weight of alcohol and 100mg/kg body weight of vitamin C after two hours, Group G received 6000mg/kg body weight of alcohol and 200mg/kg body weight of vitamin C after two hours, and Group H received 6000mg/kg body weight of alcohol and 300mg/kg body weight of vitamin C after two hours. The results show that the heart weight increased significantly in Group B (alcohol only) compared to the control group (Group A). The heart weight decreased significantly in Groups C, D, E, F, G, and H, which received vitamin C in combination with alcohol, compared to Group B. Group H had the lowest mean heart weight among all group.
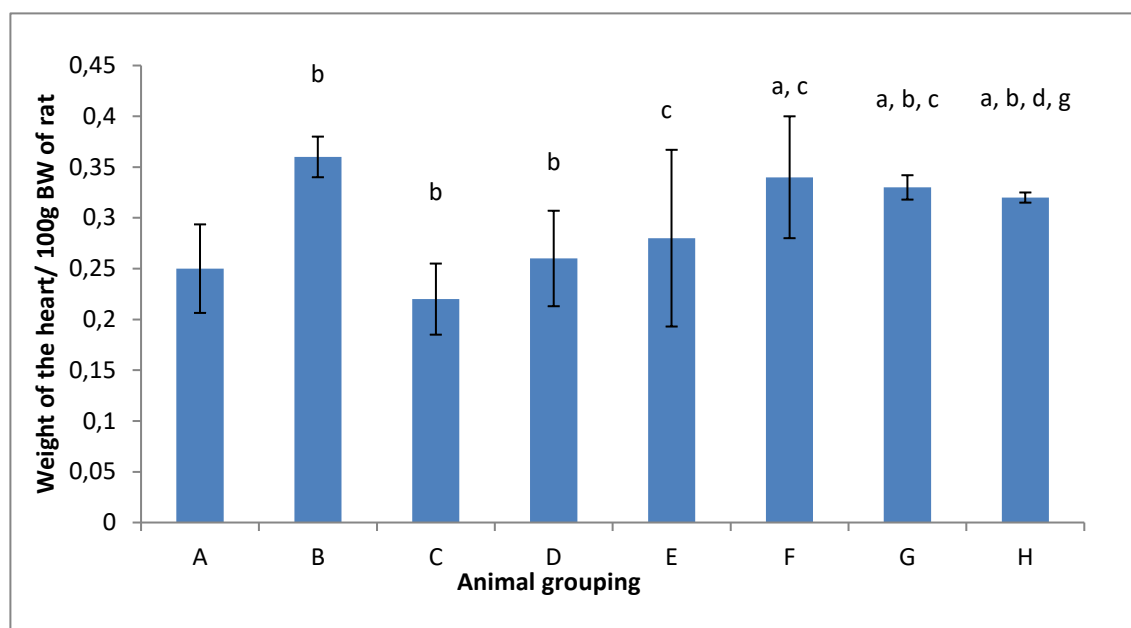
Figure 1. Ameliorative effect of vitamin C against alcohol induced weight changes in male Wistar rats.

Each value 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

Ameliorative effect vitamin C against alcohol induced oxidative stress and increase in lipid peroxidation in the heart tissue in adult male Wistar rats

The table shows the ameliorative effect of vitamin C on the antioxidant enzyme activity and level of lipid peroxidation in the heart of adult male Wistar rats. The groups were treated with different doses of alcohol and vitamin C, and the values are expressed as mean ± SEM. The results show that treatment with alcohol reduced the GSH and SOD levels and increased the MDA levels, while treatment with vitamin C increased the GSH and CAT levels. The group treated with both alcohol and vitamin C showed a significant increase in GSH and CAT levels compared to the other groups. The values were significant when compared to their respective control groups.

Table 2 Ameliorative effect vitamin C against alcohol induced oxidative stress and increase in lipid peroxidation in the heart tissue in 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	64.58±11.41	0.92±0.02	7.56±0.14	8.19±2.84
B	6000 mg/kg body weight of alcohol (30% v/v)	60.04±2.27	0.88±0.01[A]	6.58±2.05	11.65±1.95[A]
C	100 mg/kg body weight of vitamin C	63.81±3.82[B]	1.01±0.16[B]	7.59±0.97	11.33±0.45[A]
D	200 mg/kg body weight of vitamin C	71.36±10.01[B]	0.87±0,07	8.49±0.41[ABC]	8.32±3.45
E	300 mg/kg body weight of vitamin C	65.4±10.71	0.87±0.10	8.37±1.28	7.49±1.56[BC]
F	6000 mg/kg body weight of alcohol (30% v/v) and 10mg/100g body weight of vitamin C after two hours	73.57±13.53[B]	0.96±0.09[B]	6.55±2.19[DE]	7.43±0.88[B]
G	6000 mg/kg body weight of alcohol (30% v/v) and 20mg/100g body weight of vitamin C after two hours	107.64±26.39[ABCDE]	0.93±0.07	6.87±0.86[DE]	13.91±0.71[ABD]
H	6000 mg/kg body weight of alcohol (30% v/v) and 30mg/100g body weight of vitamin C after two hours	69.47±12.06[G]	1.26±0,16[ABCDEFG]	9.19±0.71[ABCDFG]	8.26±1.81[BC]

Notes: Glutathione reductase (GSH), Superoxide dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA)

[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

Ameliorative effect vitamin C against alcohol induced heart damage in adult male Wistar rats

In the histomorphology of the heart below shows that in the control group A there was a Normal cardiac muscle histology showing an organized and well differentiated cardiac fibers (red thin arrow), nucleus of cardiac fibers (black circle) and intercalated disc without any disorientation. In the heart histomorphology of the test group B, there was atrophy and interstitial fibrosis of the muscle fibers (black thin arrow), the nucleus (red circle) and myocytes necrosis (black arrow head). In the heart

histology of test group C, D and E there was no severe histomorphological changes, the nucleus (yellow circle), the cardiac muscles (black thin arrow)and the capillary layer (red thin arrow) are well organized, for test group D the nucleus (blue circle), the cardiac muscles (yellow thin arrow) and the capillary layer are also well organized, while for test group E the nucleus (black circle), the cardiac muscles (yellow thin arrow) and the capillary layer (black thick arrow) are well organized. In test group F and G there was regeneration and improvement of the cardiac fibers (black thin arrow), nucleus (red

circle) and the intercalated disc are all intact, for test group G there was regenerated and improved cardiac fibers (black thin arrow), reduced nucleus (red circle) and dilated capillary layers (black arrow head) with all morphology intact. Test group F shows well regenerated and improved cardiac fibers (blue thin arrow), slight myocytes necrosis (black arrow head) reduced nucleus (red circle) with all morphology intact.
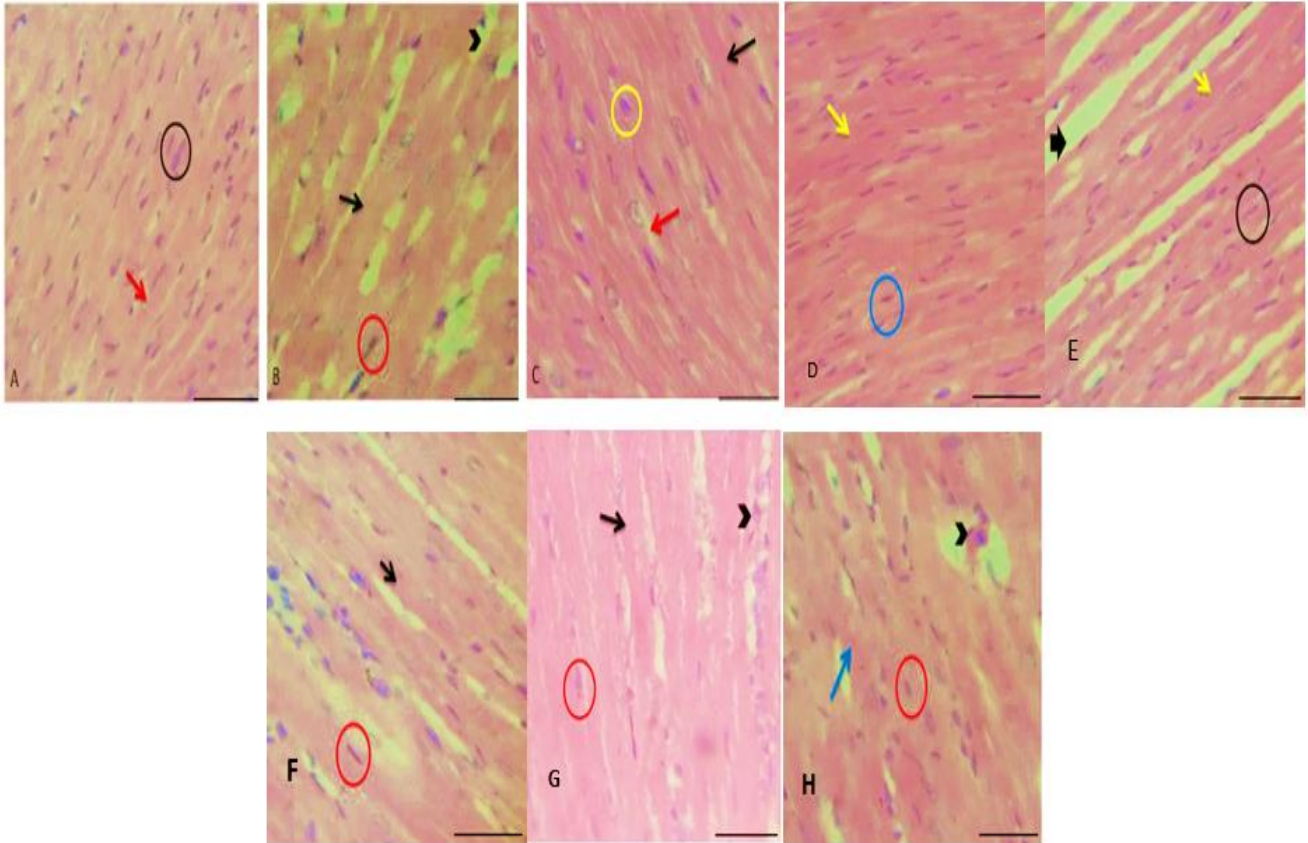

Figure 2. Ameliorative effect vitamin C against alcohol induced pathological changes in the histoarchitecture of the heart in adult male Wistar rats H/E X200. Scale Bar =120μm

A: Distilled water only

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

C: 100mg/kg body weight of vitamin C

D: 200mg/kg body weight of vitamin C

E: 300mg/kg body weight of vitamin C

F: 6000mg/kg body weight of alcohol (30% v/v) and 100mg/Kg body weight of vitamin C after two hours

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

H: 6000mg/kg body weight body weight of vitamin C after two hours

Discussion

Alcohol consumption can have various effects on the body's electrolyte balance, which is the balance of essential minerals in the blood that help regulate various bodily functions. Alcohol is a diuretic, which means that it increases urine production and can lead to dehydration. Dehydration can disrupt the balance of electrolytes in the blood, particularly sodium, potassium, and chloride ion. Alcohol consumption can affect the levels of sodium ions in the blood primarily through its effects on aldosterone secretion and dehydration. Aldosterone is produced by the adrenal glands and promotes sodium, potassium, and chloride ion reabsorption in the kidneys, which leads to an increase in blood levels of the selected electrolyte.

However, alcohol consumption can inhibit the secretion of aldosterone, leading to a decrease in blood electrolyte reabsorption and an increase in excretion of these blood electrolyte in the urine. This results in a decrease in blood electrolyte level causes, a condition known as hyponatremia (low sodium), hypokalemia (low potassium) and hypocalcemia (low calcium ion).

In addition, alcohol consumption can also cause dehydration, which can further exacerbate hyponatremia. Alcohol is a diuretic, which means it increases urine production and leads to fluid loss from the body. This fluid loss can cause a decrease in blood volume and a decrease in blood sodium

levels [19-21]. In our study the administration of alcohol lead to a decrease in the above mention blood electrolyte, this corresponds with the study of Palmer and Clegg. [22]

Electrolyte imbalances can have significant effects on the heart, as electrolytes such as sodium, potassium, and calcium are essential for proper cardiac function. Changes in the levels of these electrolytes can disrupt the normal electrical signals that regulate heart rhythm, leading to arrhythmias and other cardiac problems.

Sodium is essential for the proper functioning of the heart's electrical system. Changes in sodium levels can lead to abnormal electrical activity in the heart, which can cause arrhythmias, such as atrial fibrillation or ventricular tachycardia.

Potassium plays a critical role in regulating the heart's resting membrane potential, which is essential for normal cardiac function. Changes in potassium levels can cause alterations in the resting membrane potential, leading to arrhythmias such as bradycardia or ventricular fibrillation.

Calcium is also essential for normal cardiac function, as it regulates the strength and timing of cardiac contractions. Changes in calcium levels can lead to abnormal heart rhythms and impaired cardiac contractility. In addition to affecting the heart's electrical and mechanical function, electrolyte imbalances can also lead to structural

changes in the heart, such as myocardial fibrosis or hypertrophy, which can further impair cardiac function. [23-25]

Overall, electrolyte imbalances can have significant effects on the heart, leading to arrhythmias, impaired contractility, and structural changes as seen in our study, the administration of alcohol lead to atrophy and interstitial fibrosis of the cardiac muscle fibres. In the case of cardiac muscle fibers, atrophy can occur due to a decrease in workload or blood supply induced by oxidative stress related to alcohol consumption as seen in our study, as well as in conditions such as heart failure or myocardial infarction. Atrophy of the cardiac muscle fibres can lead to a decrease in cardiac contractility, which can impair the heart's ability to pump blood efficiently. Interstitial fibrosis refers to the accumulation of collagen fibres between the individual muscle cells in the heart. This can occur due to chronic inflammation which is mainly due to the increase in lipid peroxidation. Interstitial fibrosis can impair the mechanical and electrical properties of the cardiac muscle fibres, leading to impaired contractility and electrical conduction, as well as an increased risk of arrhythmias and heart failure. Both atrophy and interstitial fibrosis can have significant effects on cardiac function, leading to impaired contractility, abnormal electrical activity, and an increased risk of adverse cardiac events. [26-29]

Oxidative stress can have a significant impact on the structure of the heart. It occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of the body to counteract their harmful effects through antioxidant defence mechanisms. One of the ways in which oxidative stress can affect the heart structure is by inducing oxidative damage to the cardiac muscle fibres. ROS can react with lipids, proteins, and DNA in the cardiac cells, leading to structural changes and impairments in cellular function. This can lead to changes in the mechanical and electrical properties of the heart, impairing its ability to pump blood efficiently and increasing the risk of arrhythmias and heart failure. [30]

Oxidative stress can also promote inflammation in the heart, leading to the activation of immune cells and the release of inflammatory mediators. Chronic inflammation can contribute to the development of fibrosis, which is the accumulation of collagen fibres in the heart tissue. This can impair the mechanical and electrical properties of the heart, leading to impaired contractility and electrical conduction, as well as an increased risk of arrhythmias and heart failure.

Overall, oxidative stress can have a significant impact on the structure and function of the heart, leading to impaired cardiac function and an increased risk of adverse cardiac events [31]. In our study the administration of alcohol lead to decrease

in antioxidant enzymes of the heart and increase in lipid peroxidation, which is also responsible for the pathological changes seen in the heart histo-architecture and blood electrolyte level.

One of the ways in which vitamin C affects the heart is by acting as an antioxidant. Antioxidants are molecules that neutralize harmful free radicals in the body, which can cause damage to cells and tissues if left unchecked. By neutralizing these free radicals, vitamin C may help protect the heart from oxidative stress and prevent damage to the blood vessels. Vitamin C may also improve the function of the endothelium, which is the inner lining of the blood vessels. The endothelium plays a key role in regulating blood flow and blood pressure, and dysfunction of the endothelium is a common feature of many cardiovascular diseases. Studies have shown that vitamin C can improve endothelial function by increasing the production of nitric oxide, a molecule that helps to relax and dilate the blood vessels. Furthermore, vitamin C has been shown to reduce inflammation in the body, which is a risk factor for heart disease. [32-35]

In our study the administration of vitamin C have positive changes in the antioxidant enzymes activity of the heart and level of lipid-peroxidation which also lead to regenerative changes on the histology of the heart and restoration of functions, also there was improvement in the blood electrolyte level.

At 100, 200 and 300 mg/kg vitamin C has proved to have cardio- protective activity according to the result of this study, but while vitamin C is not a cure for heart disease, it may have some beneficial effects on heart function by acting as an antioxidant, improving endothelial function, and reducing inflammation. However, more research is needed to fully understand the role of vitamin C in cardiovascular health.

Authors contribution

OYESOLA, Olusoji Adebusoye: Conceptualization, methodology, resources, supervision.

OKHIAI, Peter Okhemukhokho Ohi: Methodology, resources.

ADENEKAN, Sunday Oluwaseun: Methodology, resources.

OWOEYE, Ifedolapo Ibukuoluwa Gloria: Writing, reviewing and editing.

GEORGE, Emmanuel Taiwo: Conceptualization, methodology, supervision, formal analysis, writing (original draft).

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