Ameliorative effect of Vitamin C against alcohol induced toxicity on the liver and kidney of adult male Wistar rats

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Abstract

Liver and the kidney are important organs of the body involved in the process of detoxification in the body, they play a major role in filtering and eliminating toxins and other waste from the body. Against this background, the study was undertaken to evaluate the ameliorative effect of vitamin C against alcohol induced kidney and liver toxicity in male Wistar rats. After 14 days acclimatization, 40 male Wistar rats were divided into eight groups: a control group that received only food and water. Other groups were given varying doses of alcohol, vitamin C, or a combination of both for 21 days. After, the rats were euthanized, blood, kidney and liver samples were collected to analyse for glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), level of lipid peroxidation: malondialdehyde (MDA), histology of kidney and liver tissue, urea, and creatinine levels, and liver enzymes activity. results showed that in the alcohol only group there were pathological changes in the activity of liver enzymes, increased in the urea (40.20 mg/dl) and creatinine (2.40 mg/dl) levels, high level of lipid peroxidation (15.05-24.64 µmol/ml), low SOD (1.19-1.58 µmol/ml/min/mg/pro), CAT (5.71-5.61 µmol/ml/min/mg/pro) and GSH (61.99-100.23 µmol/ml) activity, and degeneration of the liver and kidney tissue when compared to the healthy control groups. In the groups that received alcohol and vitamin C treatment there was regenerative change in the histo- architecture of the liver and kidney, reduction in the liver enzymes activity, level of lipid peroxidation and increase in the activity of SOD, GSH and CAT. In conclusion, the study provided evidence that vitamin C can protect the liver and kidney against alcohol toxicity and also improve detoxification process in the body

Keywords: Antioxidants, Ascorbic Acid, Ethanol, Kidney, Liver, Wistar rats

Effet améliorant de la vitamine C contre la toxicité induite par l'alcool sur le foie et les reins de rats mâles adultes de Wistar

Résumé

Le foie et les reins sont des organes importants du corps impliqués dans le processus de détoxification de l'organisme, ils jouent un rôle majeur dans le filtrage et l'élimination des

toxines et autres déchets de l'organisme. Dans ce contexte, l'étude a été entreprise pour évaluer l'effet améliorateur de la vitamine C contre la toxicité rénale et hépatique induite par l'alcool chez les rats mâles de Wistar. Après 14 jours d'acclimatation, 40 rats mâles de Wistar ont été divisés en huit groupes : un groupe témoin qui n'a reçu que de la nourriture et de l'eau. D'autres groupes ont reçu diverses doses d'alcool, de vitamine C ou une combinaison des deux pendant 21 jours. Après, les rats ont été euthanasiés, des échantillons de sang, de rein et de foie ont été prélevés pour analyser le glutathion (GSH), la superoxyde dismutase (SOD), la catalase (CAT), le niveau de peroxydation lipidique : malondialdéhyde (MDA), l'histologie des tissus rénaux et hépatiques. , les niveaux d'urée et de créatinine et l'activité des enzymes hépatiques. Les résultats ont montré que dans le groupe alcoolique uniquement, il y avait des changements pathologiques dans l'activité des enzymes hépatiques, une augmentation des taux d'urée (40,20 mg/dl) et de créatinine (2,40 mg/dl), un niveau élevé de peroxydation lipidique (15,05-24,64 µmol/ ml), faible activité SOD (1,19-1,58 µmol/ml/min/mg/pro), CAT (5,71-5,61 µmol/ml/min/mg/pro) et GSH (61,99-100,23 µmol/ml) et dégénérescence de les tissus hépatiques et rénaux par rapport aux groupes témoins sains. Dans les groupes ayant reçu un traitement à base d'alcool et de vitamine C, il y a eu un changement régénérateur dans l'histoarchitecture du foie et des reins, une réduction de l'activité des enzymes hépatiques, du niveau de peroxydation lipidique et une augmentation de l'activité de la SOD, du GSH et de la CAT. En conclusion, l'étude a démontré que la vitamine C peut protéger le foie et les reins contre la toxicité de l'alcool et également améliorer le processus de détoxification du corps.

Mots-clés : Antioxydants, Acide Ascorbique, Éthanol, Rein, Foie, Rats Wistar

الكبد والكلى أعضاء مهمة في الجسم تشارك في عملية إزالة السموم من الجسم يلعبان دورًا رئيسيًا في تصفية وإزالة السموم والنفايات الأخرى من الجسم في ظل هذه الخلفية، تم إجراء الدراسة لتقييم التأثير التحسيني لفيتامين ج ضد تسمم الكلى والكبد الناجم عن الكحول في ذكور فئران ويستار بعد أربعة عشر يومًا من التأقلم، تم تقسيم أربعين ذكرًا من فئران ويستار إلى ثماني مجموعات، مجموعة مراقبة لا تتلقى سوى الطعام والماء. تم إعطاء مجموعات أخرى جرعات متفاوتة من الكحول أو فيتامين سي أو مزيج من كليهما لمدة واحد وعشرين يومًا، بعد ذلك، تم القتل الرحيم للفئران، وتم جمع عينات الدم والكلى والكبد لتحليل الجلوتاثيون وفائض الأكسيد الفائق، الكاتالاز، مستوى بيروكسيد الدهون: مالونديالديهايد، أنسجة أنسجة الكلى والكبد، مستويات اليوريا والكرياتينين، ونشاط إنزيمات الكبد أظهرت النتائج أنه في مجموعة الكحول فقط كانت هناك تغيرات مرضية في نشاط إنزيمات الكبد، زادت في اليوريا (40.20 مليجرام/ديسيلتر) ومستويات الكرياتينين (2.40 ملغم/ديسيلتر)، مستوى عالٍ من بيروكسيد الدهون (15.05-24.64 ملليمتر/مل)، الكبد والكلى أعضاء مهمة في الجسم تشارك في عملية إزالة السموم من الجسم يلعبان دورًا رئيسيًا في تصفية وإزالة السموم والنفايات الأخرى من الجسم في ظل هذه الخلفية، تم إجراء الدراسة لتقييم التأثير التحسيني لفيتامين ج ضد تسمم الكلى والكبد الناجم عن الكحول في ذكور فئران ويستار بعد أربعة عشر يومًا من التأقلم، تم تقسيم أربعين ذكرًا من فئران ويستار إلى ثماني مجموعات، مجموعة مراقبة لا تتلقى سوى الطعام والماء. تم إعطاء مجموعات أخرى جرعات متفاوتة من الكحول أو فيتامين سي أو مزيج من كليهما لمدة واحد وعشرين يومًا، بعد ذلك، تم القتل الرحيم للفئران، وتم جمع عينات الدم والكلى والكبد لتحليل الجلوتاثيون وفائض الأكسيد الفائق، الكاتالاز، مستوى بيروكسيد الدهون: مالونديالديهايد، أنسجة أنسجة الكلى والكبد، مستويات اليوريا والكرياتينين، ونشاط إنزيمات الكبد أظهرت النتائج أنه في مجموعة الكحول فقط كانت هناك تغيرات مرضية في نشاط إنزيمات الكبد، زادت في اليوريا (40.20 مليجرام/ديسيلتر) ومستويات الكرياتينين (2.40 ملغم/ديسيلتر)، مستوى عالٍ من بيروكسيد الدهون (15.05-24.64 ملليمتر/مل)،انخفاض الأكسيد الفائق (1.19-1.58 µmol/ml/min/mg/pro) كتالاز (5.71-5.61 µmol/ml/min/mg/pro) الغلوتاثيون (61.99-100.23 µmol/ml) في الحركة. تنكس أنسجة الكبد والكلى عند مقارنتها بمجموعات التحكم الصحية في المجموعات التي تلقت علاج الكحول وفيتامين سي، كان هناك تغيير تجديدي في البنية النسيجية للكبد والكلى انخفاض نشاط إنزيمات الكبد، ومستوى تفوق أكسدة الدهون زيادة في حركة الديسموتاز والغلوتاثيون وكتالاز في الختام، قدمت الدراسة دليلاً على أن فيتامين سي يمكن أن يحمي الكبد والكلى من تسمم الكحول ويحسن أيضًا عملية إزالة السموم في الجسم.

Introduction

Consumption of alcohol is responsible for a high number of preventable deaths worldwide, with three million fatalities attributed to it annually. It is widely consumed across the globe, with most individuals drinking it regularly (Witkiewitz *et al*., 2019). Although alcohol is classified as a sedative-hypnotic drug, which depresses the central nervous system at high doses (Milhorn, 2018), it can act as a stimulant at lower doses, leading to feelings of talkativeness and euphoria. However, excessive alcohol intake can result in drowsiness, respiratory depression, coma, or even death. Moreover, alcohol consumption is linked to more than 200 diseases, injuries, and other health conditions. It is associated with the development of mental and behavioral disorders, including alcohol addiction, as well as major non-communicable diseases such as liver cirrhosis, alcoholic kidney diseases, some cancers, and cardiovascular disease (Marshall, 2014; Shield *et al*., 2013).

Vitamins have several unique characteristics that distinguish them from other essential biological compounds, such as proteins, carbohydrates, and lipids. While animals can synthesize these substances in sufficient quantities, vitamins are generally unable to be synthesized in adequate amounts to meet the body's needs and must be obtained from the diet or synthesized synthetically (Doseděl *et al*., 2021). For this reason, vitamins are known as essential food nutrients. Water-soluble vitamins include vitamin C (ascorbic acid) and the B vitamins, such as thiamin (vitamin B1), riboflavin (vitamin B2), vitamin B6, niacin (nicotinic acid), vitamin B12, folic acid, pantothenic acid, and biotin. These vitamins are relatively simple molecules composed of carbon, hydrogen, and oxygen, with some containing nitrogen, sulphur, or cobalt (Godswill *et al*., 2020). In contrast, fat-soluble vitamins include vitamins A, D, E, and K. Vitamin C a water-soluble vitamin serves as an antioxidant an essential co-factor for collagen biosynthesis, carnitine and catecholamine metabolism, and dietary iron absorption. It is found in citrus fruits, berries, tomatoes, potatoes, and green leafy vegetables (Doseděl *et al*., 2021).

Alcohol can cause oxidative stress, which has led to the use of antioxidant compounds to protect against liver and kidney injury. Animal studies have shown that various compounds with antioxidant properties can be protective against alcohol-induced malfunction (Cheng *et al*., 2014; Wang *et al*., 2012). Antioxidant therapy has been considered a potential treatment option for several diseases, including alcoholic liver and kidney disease. In rats, antioxidants such as glutathione, vitamin E, and 2-hydroxy-4-methoxy benzoic acid have been found to protect against alcohol-induced liver injury (Guo *et al*., 2013). The present study aims to evaluate the ameliorative activity of vitamin C against alcohol-induced toxicity in the liver and kidney.

Material and methods

Animal care and grouping

For this experiment, 40 adults 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 internationally recognized standard rules for the use of animals were followed in the handling and care of the

animals (National Research Council, 2011).

Ethical approval for th**e 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.

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 kidney and liver

The liver and kidney tissues to be assessed for oxidative studies were homogenized in phosphate buffer in ratio 4:1. Glutathione reductase (GSH) activities were determined using the method described by Sedlak and Lindsay, (1968), malondialdehyde (marker of lipid peroxidation (MDA) was determined using the method described by Buege and Aust, (1978), catalase (CAT) activities were determined using method described by Sinha (1972) and Superoxide dismutase (SOD) activities were determined by method described by Sun and Zigman (1978).

Urea level determination

Urea was assayed according to the method described in Randox urea kits. 2.50 mL of reagents 1, 2 and 3 were added to 10 micro litter of plasma sample and were mixed immediately and incubated at 37°C for 10 minutes. The absorbance of plasma sample and standard were measured against the blank at 546 nm.

Creatinine level determination

Creatinine was assayed according to the method described in Randox kits. 2.0mL of working reagent was added to 0.2 mL of plasma. This was measured against the blank containing standard solution. It was mix and after 30 seconds, the absorbance of A1 of the standard and plasma were read at 492 nm. Exactly 2 minutes later, absorbance of A2 of standard and plasma were read at 492 nm.

Determination of liver function parameters

The activity of aspartate aminotransferase (AST) was determined by kinetic method as reported by Young, (1997). The alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were assayed by colorimetric endpoint method as described by Young *et al*. (1975).

Determination of total protein, total and conjugated bilirubin concentration

Total protein and its components were determined according to the methods of Cheesbrough (Cheesbrough, 2006), bilirubin concentration was determined according to the method of Tietz (Tietz, 2000)

Histological examination

After, harvesting the kidney and liver 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 heamatoxylin and eosin using standard procedures. The slides were viewed under light microscope and photomicrographs were taken (200×)

Statistical analysis

All the values are expressed as mean ± standard error of mean (SEM). Analysis of data was done using GraphPad Prism version 5 for Windows. Differences between groups were analyzed by one-way ANOVA followed by Student's Newman-Keuls post-hoc test. Differences were considered significant when $P < 0.05$

Results

Ameliorative activity of vitamin C against alcohol induced pathological changes on liver enzymes activity in adult male wistar rats

Observation from Table 1 howed the effect of the concurrent administration of alcohol and vitamin C on liver enzymes activity in male wistar rats. In the serum liver enzyme activity, groups administered with alcohol only showed significant increase in the AST and ALP activity, there was also increase in ALT activity, but this increase was not significant. Across groups administered with vitamin C only, there was decrease in the liver enzyme activity when compared to both the control group and the alcohol only group. In the co- administration groups, there was decrease in the liver enzyme activity when compared to the alcohol only group and significant improvement was also noticed when compared to the normal control group and the vitamin C only groups.

Table 1 Ameliorative activity of vitamin C against alcohol induced pathological changes on liver enzymes activity in adult male wistar rats

Groups	AST (mg/L)	ALT (mg/L)	ALP (mg/L)
A	17.20±0.84	10.00.00±1.00	18.60±1.14
B	23.40±1.30^{A}	13.60±2.41	27.20±0.55^{A}
C	16.40±1.14^{A}	9.20±0.84^{A}	19.8±3.11
D	15.20±2.1$^{B, C}$	8.80±1.30^{C}	17.6±1.14^{B}
E	15.80±0.83^{A,C,D}	7.80±1.30^{A,C,D}	17.6±0.89^{D}
F	20.40±0.54^{A,C,D}	11.60±0.54^{A,C,D}	22.2±3.42^{E}
G	19.40±0.54^{A,D,E,F}	11.00±1.00^{B,C,E,F}	20.6±0.54^{B,E}
H	18.40±0.54^{A,C,D,G}	9.80±1.30^{A,C,D,G}	19.20±1.1^{A,B,C,D,E,F,G}

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 activity of vitamin C against alcohol induced pathological changes on

the total protein, total and conjugated bilirubin, urea and Creatinine levels in male wistar rats

In Table 2 are total protein and albumin content and were reduced significantly in group administered with alcohol only when compared to the control group. In groups administered with vitamin C only, there was improvement in the total protein and albumin content when compared to the control group and increased when compared to the alcohol only group. Significant increase was noticed in the total protein albumin content in groups administered with alcohol and vitamin C; also, there was improvement when compared to the normal control and vitamin C only groups. The bilirubin content in rats administered with alcohol only increased significantly when compared to the normal control group, across the vitamin C only groups there was decrease in the bilirubin level when compared to the normal control and the alcohol only group., reductions was also seen in the co- administration groups when compared to the alcohol only group, also positive changes was noticed when the co- administration group was compared to the vitamin only groups and the control group. Alcohol intake at 6000 mg/kg body weight led to significant increases in urea and creatinine levels (Group B). Treatment with vitamin C at 100 mg/kg body weight significantly reduced urea levels (Group C), while treatment with vitamin C at 300 mg/kg body weight significantly reduced creatinine levels (Group H). When vitamin C was administered 2 hours after alcohol intake, both urea and creatinine levels were reduced (Groups F, G, and H).

Table 2 Ameliorative activity of vitamin C against alcohol induced pathological changes on the total protein, total and conjugated bilirubin, urea and Creatinine levels in male wistar rats

Groups	TP (mg/dL)	ALB (g/dL)	TB (µmol/L)	CB (µmol/L)	Urea (mg/dl)	Creatinine (mg/dl)
A	7.2±0.62	3.12±0.38	0.32±0.13	0.28±0.13	34.40 ±6.95	1.20 ±0.16
B	4.32±0.15 [A]	1.16±0.05 [A]	0.52±0.13 [A]	0.54±0.05 [A]	40.20 ±5.97 [A]	2.40 ±0.58
C	7.86±0.07 [B]	3.2±0.29 [A]	0.28±0.05 [A]	0.16±0.05 [A]	13.00 ±3.94 [A, B]	1.20 ±0.20 [B]
D	8.50±0.20 [A,B,C]	3.22±0.30 [B,C]	0.24±0.08 [C]	0.16±0.05 [A]	27.80 ±20.66	1.16 ±0.05 [B]
E	8.50±0.54 [A,C,D]	3.22±0.40 [A,D]	0.24±0.05 [C]	0.13±0 [B]	14.60 ±3.58 [A, B]	1.14 ±0.13 [B]
F	5.42±0.59 [A,C,E]	1.96±0.65 [B,C, E]	0.34±0 [B,C,D,E]	0.3±0 [B,C, D,E]	24.80 ±3.03 [A, B, C, E]	1.40 ±0.14 [A, B, D, E]
G	5.92±0.51 [A,C]	1.96±0.05 [B,C,E]	0.32±0.05 [C, F]	0.14±0.05 [A,E,F]	20.60 ±3.65 [A, B, C, E, F]	1.64 ±0.21 [A, B, C, D, E, F]
H	6.74±0.15 [A,B,C,D,E,]	2.46±0.05 [A,B,C,D,E,F,G]	0.31±0.04 [C,F]	0.14±0.05 [B,C D,F,G]	19.80 ±3.19 [A, B, C, E, F]	1.38 ±0.41 [B]

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 activity of vitamin C against alcohol induced oxidative stress and increase in the level of lipid peroxidation in the liver of adult male wistar rats.

Table 3 shows the effect of the concurrent administration of alcohol and vitamin C on antioxidant enzyme activity and lipid peroxidation level in the liver of male wistar rats. Group administered with alcohol only showed depletion in SOD, CAT and GSH activity of the liver, viatamin C only groups showed significant increase in the antioxidant enzymes activity when compared to the control group. In the concurrent administration group, there was significant improvement in the liver antioxidants enzyme activity when compared to the control group and the vitamin C only group. In rats administered with alcohol only there was toxic increase in the level of lipid peroxidation when compared to the control group, in the vitamin C only groups the level of lipid peroxidation reduced when compared to the control group and the alcohol only group, these positive changes was also noticed in the co- administration groups as there were positive changes noticed in the level of lipid peroxidation after the administration of vitamin C.

Table 3 Ameliorative effect activity of vitamin C against alcohol induced oxidative stress and increase in the level of lipid peroxidation in the liver of adult male wistar rats

Groups	GSH (µmol/mL)	SOD (µmol/ml/min/mg/pro)	CAT (µmol/ml/min/mg/pro)	MDA (µmol/ml)
A	202.88±10.02	1.69±0.07	6.73±0.75	15.34±3.89
B	100.23±6.77^{A}	1.58±0.20	5.61±0.31^{A}	26.24±3.51
C	254.55±33.92^{B}	2.27±0.21^{A}	8.67±0.33$^{A, B}$	14.24±3.03
D	280.95±18.61$^{A, B}$	2.37±0.13$^{A, B}$	9.12±1.04$^{A, B}$	12.15±4.85
E	335.56±20.52$_{A, B}$	2.59±0.17$^{A,C D}$	10.74±0.39$^{A, C, D}$	15.00±0.59
F	181.64±26.63$^{A, C D, E}$	2.06 ± 0.18$^{A, B, E}$	7.80 ±1.15$^{B, D,E}$	12.40 ±5.26
G	242.73 ±48.79^{B,CDEF}	2.15 ±0.51^{A,B,E}	7.08 ±1.56^{B,C,D}	17.71 ±3.04$^{D, E,F}$
H	287.10 ±15.29 $^{A, C, D, E, G}$	7.73 ±7.55	6.08 ±1.62^{C,D,F}	15.69 ±4.28^{A,B,C,D,E,F,G}

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 activity of vitamin C against alcohol induced oxidative stress and increase in the level of lipid peroxidation in the kidney of adult male wistar rats

The results in table 4 below showed that the antioxidant enzymes' activity and lipid peroxidation levels varied significantly between the different groups. Test Group B had significantly increase in MDA activity and decrease in SOD, CAT and GSH activity when compared to the control group A, while groups C and D had significant differences in the CAT, GSH and SOD activity when compared to groups A and B. Group E had significant differences in the CAT, GSH and SOD activity when compared to groups A, B, C, and D. Also group F had significant differences in the CAT, GSH and SOD activity when compared to groups A, B, C, D, and E. Group G had significant differences compared to groups A, B, C, D, and E in the MDA, CAT, GSH and SOD activity, while group H had significant differences compared to groups A, B, C, E, and G E in the MDA, CAT, GSH and SOD activity.

Table 4 Antioxidant enzymes activity (CAT, SOD, and GSH) and level of lipid peroxidation (MDA) in male wistar rats.

Group	GSH (µmol/ml)	SOD (µmol/ml/min/ mg/pro)	CAT (µmol/ml/min/ mg/pro)	MDA (µmol/ml)
A	211.25±19.28	3.28±0.57	17.45±4.67	13.48±1.07
B	61.99±7.31 A	1.19±0.07 A	5.71±0.23 A	15.05±3.30
C	215.69±4.52 B	2.77±0.20 B	13.18±2.0 B	11.61±2.95
D	186.99±14.25 A, C	2.95±0.17 B	17.55±1.06 C	10.05±3.77 A
E	56.19±9.93 A, B, C, D	2.34±0.33 A, B, C, D	11.95±1.64 A, B, D	8.44±1.73 A, B, C
F	55.97±0.61 A, B, C, D	1.83±0.41 A, B, C, D, E	9.23±3.32 A, B, C, D	10.09±1.54 A
G	150.95±26.77 A, B, C, D, E, F	1.34±0.07 A, B, C, D, E, F	6.85±0.27 A, B, C, D, E	16.76±2.61 A, B, C, D, E, F
H	178.72±4.58 A, B, C, E, F, G	3.25±0.13 B, C, D, E, F, G	17.14±0.55 B, C, E, F, G	10.38±1.22 A, B, E, G

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 activity of vitamin C against alcohol induced pathological changes on the histo- architecture of liver of adult male wistar rats

Plate 1 shows the effect of vitamin on alcohol induced toxicity on the liver, the Control group showed normal

histomorphology of liver with well-defined hepatocytes (black circle), central vein (black thick arrow) well differentiated sinusoid (red thin arrow) and well-organized hepatic plate (red arrowhead). In rats treated with 6000 mg/kg alcohol there was serious liver degeneration and irregularities in the diameter of central vein (red thick arrow), hepatic plates (black thin arrow), with constricted sinusoids and reduction in the number of kupfer cells (red arrowhead) and undifferentiated hepatocytes. In groups treated with 100 mg/kg, 200 mg/kg and 300 mg/kg of commercial grade vitamin C, there was no significant histomorphological changes, the central vein (blue thick arrow), hepatocytes (red circle) are well differentiated, reduced and dilated sinusoids with kupfer cells (black thin arrow) and hepatic plates (red thin arrow) were seen in the liver histology. In the liver histology of rats treated with 6000 mg/kg alcohol and 100 mg/kg and 200 mg/kg of commercial grade vitamin C concurrently, there was slight regeneration of the tissue histomorphology, with congested central vein (black thick arrow), well differentiated hepatocytes (red circle) without steatosis, constricted sinusoids with kupfer cells (red thin arrow) and the hepatic plate (red thin arrow) are intact without any loss of function. In rats treated with 6000 mg/kg alcohol and 300 mg/kg commercial vitamin C there was regeneration of the tissue histomorphology, Central vein (black thick arrow) well differentiated hepatocytes(red circle) without hepatosteatosis, dilated sinusoids with kupfer cells(blue thin arrow) and the hepatic plate (black thin arrow) are intact and well differentiated without any loss of function.

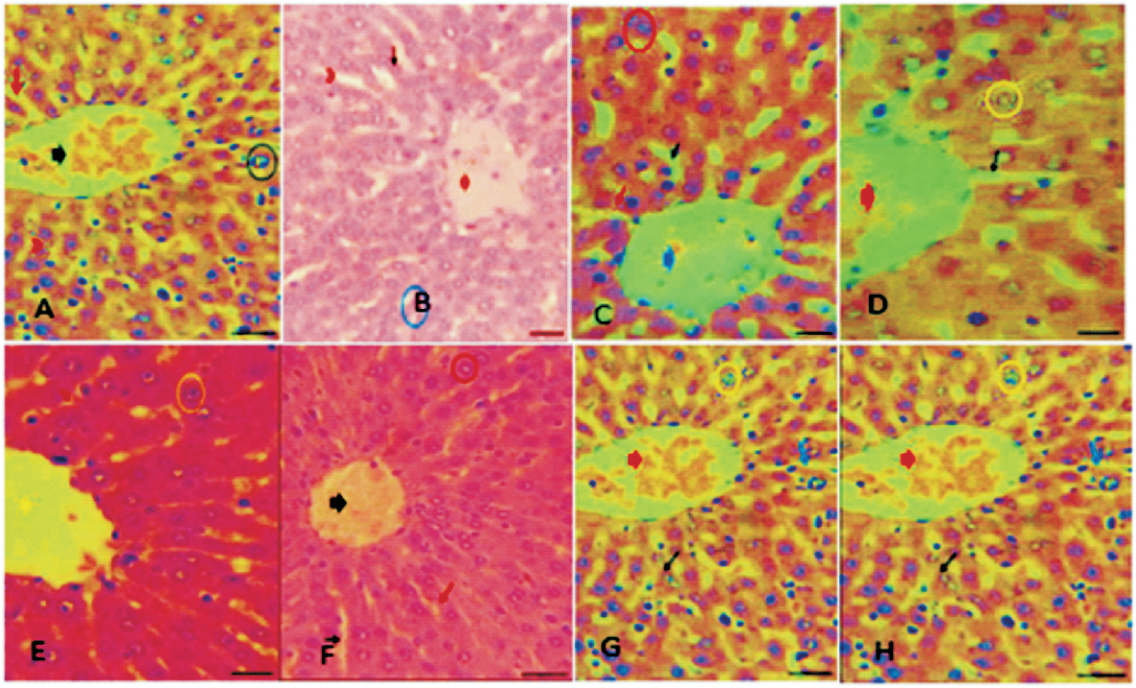

Plate 1, Ameliorative activity of vitamin C against alcohol induced pathological changes on the histo-architecture of liver of adult male wistar rat's H/E X200. Scale Bar =120µm.

Ameliorative activity of vitamin C against alcohol induced pathological changes on the histo- architecture of kidney of adult male wistar rats

The image presented (Plate 2) illustrates the impact of concurrent administration of alcohol and vitamin C on the kidney's histoarchitecture. The control group showed a distinct renal capsular space (CS), proximal tubules (PCT), distal proximal tubules (PCT), and well-differentiated epithelial cells (indicated by a black thin arrow). In the group that received only alcohol, degeneration and irregularities of the glomerulus and capsular space (CS) were observed, with constricted proximal and distal tubules (PCT and DCT) and a reduced number of epithelial cells (indicated by a red thin arrow). The group that received only vitamin C showed constriction and thickness of the glomerulus (indicated by a red thick arrow), as well as the proximal and distal tubules (PCT and DCT), with a well-differentiated capsular space (CS), and a well-differentiated glomerulus (indicated by a yellow thick arrow) without any loss of function. In the group that received 6000 mg/kg of alcohol and 100 mg/kg of vitamin C, there was slight regeneration of tissue histomorphology, with congested distal and proximal tubules (DCT and PCT), a glomerulus with podocytes (indicated by a black thick arrow), normal capsular spaces (CS), and intact epithelial cells (indicated by a red thin arrow) without any loss of function. In the group that received 6000 mg/kg of alcohol and 200 mg/kg of vitamin C, there was tissue histomorphology regeneration, with a well-differentiated capsular space (CS), podocytes on the glomerulus (indicated by a red thick arrow), and well-differentiated proximal and distal tubules (PCT and DCT) and epithelial cells (indicated by a red thin arrow) without any loss of function. Finally, in the group that received 6000 mg/kg of alcohol and 300 mg/kg of vitamin C, there was tissue histomorphology regeneration, with a well-differentiated capsular space (CS), podocytes on the glomerulus (indicated by a yellow thick arrow), and well-differentiated proximal and distal tubules (PCT and DCT) and epithelial cells (indicated by a red thin arrow) without any loss of function.

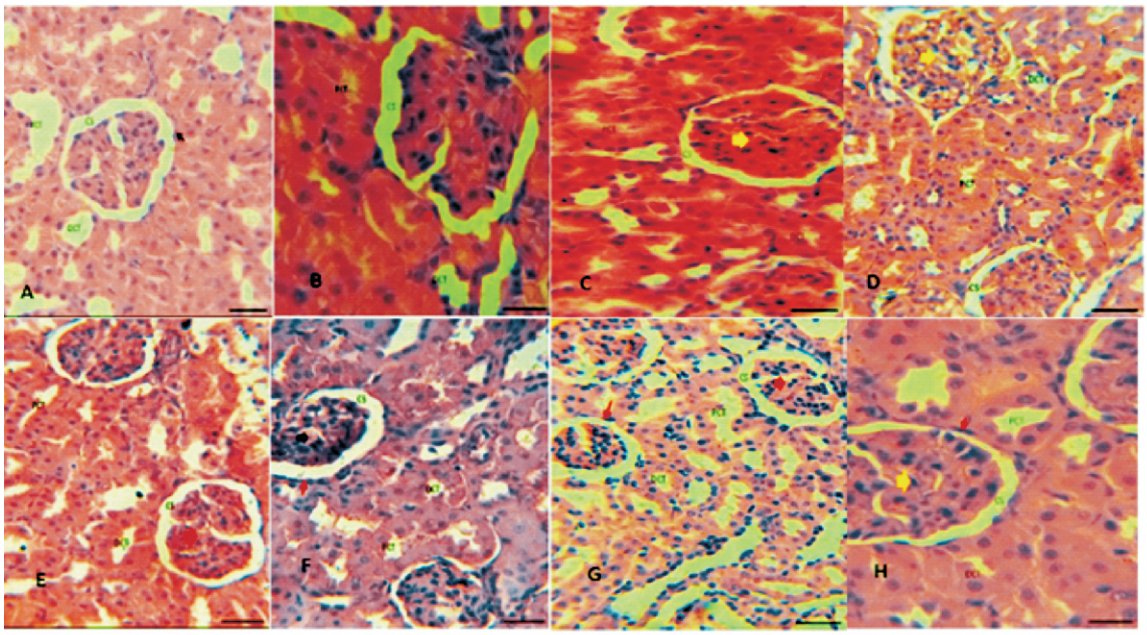

Plate 2, Ameliorative activity of vitamin C against alcohol induced pathological changes on the histo-architecture of kidney of adult male wistar rats H/E X200. Scale Bar =120µm

Discussion

After consumption, most alcohol molecules can be oxidatively metabolized to acetaldehyde and then irreversibly converted to acetate in numerous tissues, including the liver, stomach, and possibly brain at very low levels" (Ballway and Song, 2021). However, the primary site of oxidative alcohol metabolism occurs in the hepatocytes of the liver (Nassir, 2014). Alcohol metabolism generates products that damage the liver and other organs including the kidney, resulting in alcoholic liver disease (ALD, a main cause of chronic liver disease (Osna *et al*., 2017), which is characterized by a decrease in NAD+ levels, coupled with an increase of NADH levels, which will affect the kidney function. This causes redox changes and alters numerous cellular functions, resulting in elevated fat synthesis, decreased fat oxidation, and increased cell death processes (Zakhari, 2013; Zhu *et al*., 2012). According to the result of this study, there was an increase in the level of lipid peroxidation in both the liver and kidney. This is due to the fact that the oxidative metabolism of alcohol leads to the accumulation of lipid in the hepatocyte. When there is lipid peroxidation, the cellular integrity is affected because the cellular membrane is mainly composed of lipid (Xiao *et al*., 2017). There is a loss of membrane fluid and the disruption of normal membrane function. This is also seen in Table 1, as the consumption of alcohol led to an increase in serum activities of liver enzymes. This can lead to cellular dysfunction, decreased signal transduction, and ultimately, cell death, as seen in Plate 1b, in which the consumption of alcohol led to degeneration and death of the liver cells. The increase in the level of lipid peroxidation can also result in the production of harmful products, such as malondialdehyde, which can further contribute to cellular damage and cellular dysfunction. The increase in the level of lipid peroxidation is also responsible for the depletion of GSH (Recknagel *et al*., 2020). The source of ROS in hepatocytes is the microsomal cytochrome P-450 system. Chronic ethanol consumption has also been shown to stimulate microsomal ROS generation as a result of the induction of cytochrome P-450 2E1 (CYP2E1) and uncoupling between CYP2E1 and NADPH cytochrome C reductase (Lu *et al*., 2012) The elevation of ethanol-induced CYP2E1 activity is suggested to be a major contributor to generating a state of oxidative stress, resulting in the depletion of CAT and SOD enzyme activity. The depletion of CAT and SOD has been linked with an increase in inflammation (Suriyaprom *et al*., 2019).

An increase in urea and creatinine levels in the blood can be indicative of kidney dysfunction or damage. Urea and creatinine are waste products that are normally filtered out of the blood by the kidneys and excreted in urine. If the kidneys are not functioning properly, these waste products can build up in the blood, leading to high levels of urea and creatinine (Brisco *et al*., 2013; Selewski and Symons, 2014; Suriyaprom *et al*., 2019). In our study the consumption of alcohol lead to an increase in the blood level of urea and creatinine. Alcohol consumption can increase urea and creatinine levels by causing dehydration, which in turn can lead to kidney dysfunction (Penning *et al*., 2010). Alcohol is a diuretic, meaning that it increases urine output and can cause dehydration. Dehydration reduces blood flow to the kidneys, which can impair their ability to filter waste products like urea and creatinine from the blood (García-Arroyo *et al*., 2016; Watson *et al*., 2015). As a result, urea and creatinine levels in the blood can increase. Chronic alcohol consumption can also cause damage to the kidneys over time,

leading to kidney dysfunction and an increase in urea and creatinine levels. This can occur as a result of direct toxicity to the kidney tissue, or due to the development of conditions like alcoholic liver disease or high blood pressure, which can contribute to kidney damage (Remington *et al*., 2016). Additionally, the decrease in total protein levels in the groups administered with alcohol alone is also consistent with changes in albumin levels. This may be due to the inhibition of albumin biosynthesis through specific enzymes involved in cell processes and the low excretion of hormones that regulate protein biosynthesis (Ibrahim *et al*., 2012). Alcohol can precipitate soluble protein and albumin in plasma, which can be used as carriers for metabolic products of alcohol. Moreover, alcohol may inhibit protein biosynthesis through specific enzymes in cell processes and low excretion of hormones such as T3 and T4, ultimately leading to reduced production of total protein and its components in the body.

The glomerulus is a network of small blood vessels located in the kidney. Its primary function is to filter waste products and excess fluid from the blood, which then pass into the capsular space, a hollow area that surrounds the glomerulus (Molema and Aird, 2012). Degeneration and irregularities of the glomerulus and capsular space can occur due to inflammatory conditions such as glomerulonephritis can cause damage to the glomerulus, leading to degeneration and irregularities. Degeneration and irregularities of the glomerulus and capsular space can impair the kidney's ability to filter waste products and excess fluid from the blood. This can lead to a build-up of toxins and waste products in the body as seen in the increase of urea and creatinine. Excessive alcohol consumption can lead to damage to the kidneys, including inflammation and scarring of the glomerulus and capsular space. This damage can reduce the kidneys' ability to filter waste and excess fluid from the blood, leading to conditions such as proteinuria (the presence of excess protein in the urine) and oedema (swelling) in various parts of the body. Alcohol can also increase blood pressure, which can further damage the glomerulus and capsular space, leading to chronic kidney disease (Agarwal and Werner Goedde, 1990).

Alcohol induce toxicity through inflammation and reducing the level of antioxidant present in cell. Vitamin C on the other hand is a water-soluble vitamin that plays a crucial role in several biological processes in the body. One of its primary functions is as an antioxidant, which means it helps to protect cells and tissues from damage caused by free radicals. Free radicals are highly reactive molecules that can damage cells and contribute to the development of several diseases. Antioxidants such as vitamin C neutralize free radicals by donating an electron to stabilize them and prevent them from causing damage. Vitamin C has several antioxidant mechanisms (Hamid *et al*., 2010). It can directly neutralize free radicals by donating an electron to stabilize them. It can also regenerate other antioxidants, such as vitamin E, that have already donated an electron to neutralize free radicals. Additionally, vitamin C can participate in the production of enzymes that help to break down and remove free radicals from the body. Also, vitamin C has been shown to have anti-inflammatory properties in the body. Inflammation is a natural response of the immune system to injury or infection, but chronic inflammation can contribute to the development of several diseases, one of the

mechanisms by which vitamin C exerts its anti-inflammatory effects is by inhibiting the production of pro-inflammatory cytokines, which are signaling molecules that play a key role in the inflammatory response (Elsayed Azab *et al*., 2019). Vitamin C can also reduce the activation of immune cells that produce pro-inflammatory cytokines, such as macrophages and T cells. Vitamin C also has a role in modulating the production of nitric oxide (NO), which is a molecule involved in the inflammatory response. Vitamin C can regulate the production of NO, and in doing so, it can help to reduce inflammation (Bae and Kim, 2020). The protective function of vitamin C can be seen in plate 1 and 2 (F- H), where there was regeneration and restoration of function in the kidney and liver histology, also there was reduction in oxidative stress as there was increase in SOD, CAT and GSH activity of the kidney and liver and reduction in level of lipid peroxidation, also the administration of vitamin C led to a decrease in liver enzymes activity and increased the protein content of the body. Vitamin C also played detoxification function as there was reduction in the level of urea and creatinine in alcohol vitamin C groups.

Conclusion

According to the study, vitamin C had anti-inflammatory, antioxidant and detoxification properties that were mediated through several mechanisms, including the inhibition of pro-inflammatory cytokines, modulation of Nitric oxide (NO) production, and reduction of oxidative stress. These properties make vitamin C a potential therapeutic agent for the prevention and treatment of alcohol induced toxicity

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