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Commercial Grade Vitamin C Protection Against Alcohol-Induced Lipid Peroxidation

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Abstract: Alcohol is a psychoactive substance that provides energy, but it is also responsible for the development of numerous diseases. The main aim of this study is to access the protective effect of vitamin C on alcohol induced lipid peroxidation and dyslipidaemia in adult male Wistar rats. Forty male Wistar rats weighing between 150g-250g were randomly grouped into four groups of five rats each after a fourteen days acclimatization period. The control group received food and water only, test group B received 6000 mg/kg of alcohol, group C received 100 mg/kg of vitamin C, group D received 200 mg/kg of vitamin C, group E received 300mg/kg of vitamin C, group F received 6000 mg/kg of alcohol and 100 mg/kg of vitamin C, group G received 6000 mg/kg of alcohol and 200 mg/kg of vitamin C, and group H received 6000 mg/kg of alcohol and 300 mg/kg of vitamin C. The administration was done for twenty-one days, blood was collected form he ocular vein and analysed for level of lipid peroxidation and fasting lipid profile, using standard procedure. The results showed that alcohol increased the level of lipid peroxidation, low density lipoprotein, cholesterol and triglycerides, and decreased the level of high-density lipoprotein. However, in the co-administration groups, positive changes were noticed in the level of lipid peroxidation, LDL, cholesterol, and triglycerides, and there was an improvement in the level of HDL. Positive changes were also seen in the groups administered with different doses of vitamin C. The study's findings suggest that vitamin C may be useful in managing alcohol-induced toxicity since it had a favourable effect on lipid profile and peroxidation.

Keywords: Alcohol, Lipid Peroxidation, Lipid Profile, Vitamin C

1. Introduction

Alcohol is a psychoactive drug, which also provides energy; alcohol consumption is responsible for the development of several diseases [1]. It is known that alcohol causes oxidative stress and compromises the nutritional status. Patients with chronic alcohol abuse are malnourished, this may be due to the reduced intake of essential nutrient or due to the low absorption rate of essential nutrient caused by alcohol [2]. Additionally, the metabolic pathways for ethanol themselves produce intermediate toxic metabolites (free radicals and acetaldehyde) that will lead to the disruption of the normal metabolism of vital substance, causing cellular damage via oxidation mechanisms and secondary oxidative stress inflammation [3]. Alcohol is widely consumed around the world, and it can have both good and bad effects. Despite the fact that numerous studies have found a link between light to moderate alcohol use and a reduced risk of cardiovascular death [4-5]. All the same, alcohol is the most frequently consumed psychoactive substance in Nigeria among both adults and adolescents [6]. People who consume alcohol, over time start to develop poorer health. According to various studies alcohol consumption increases the risk of developing deleterious diseases such as cancer, tuberculosis, liver cirrhosis and pneumonia [7-8]

Vitamins are essential in the human body for several biochemical and physiological processes. However, since the body does not generate most vitamins, they must be obtained through diet [9]. Although ascorbic acids serve as the representative of all these molecules, vitamin C also goes by the name hexuronic acid, cevitáminic acids and xiloascórbic acid [10]. Although its function is still unclear at the cellular level, ascorbic acids plays a significant role in the human body. Collagen synthesis depends on it since the protein serves a variety of connective roles in the

body. The synthesis of hormones and neurotransmitters, as well as the metabolism of specific amino acids and vitamins also depends on ascorbic acids. Moreover, it plays a very significant role in the detoxification of the liver of harmful compounds and blood level for immunity. In order to lessen the symptoms of inflammation, an antioxidant reacts with histamine and peroxide [11-13]. In addition, vitamin C metabolism is highly regulated in healthy individuals, resulting in a complex relationship between the steady-state levels of many physiological systems and tissues [14].

Lipid peroxidation is a chemical process that occurs when free radicals attack lipids (fatty acids) in cell membranes, causing them to break down and produce harmful compounds. When this process occurs in the arteries, it can lead to atherosclerosis, a condition in which the arteries become narrowed and hardened due to the buildup of plaque [15]. The breakdown products of lipid peroxidation can be toxic to cells and cause inflammation, which can trigger the development of atherosclerosis. These toxic products can also promote the accumulation of cholesterol in the arterial walls, leading to the formation of plaque [16]. The prevention of alcohol induced lipid peroxidation and dyslipidemia will be assessed in this study

2. Material and Methods

2.1 Animal Care and Grouping

For this experiment, forty (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 [17].

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

2.2 Procedure for blood collection

The rat was restrained, the neck was gently scruffed and the eye was made to bulge before blood was drawn from the orbital venous sinus. Blood was allowed to flow into a plain sample container by capillary action through a capillary tube that was inserted dorsally into the eye

2.3 Determination of Fasting Lipid Profile

using standard techniques, the collected blood was screened for serum triglycerides, cholesterol, high density lipoprotein (HDL), cholesterol, and low-density lipoprotein (LDL)

2.4. Procedure for determination of serum level lipid peroxidation

malondialdehyde, a marker of lipid peroxidation (MDA), was measured using the Buege and Aust [18], after the blood sample to be evaluated for level of lipid peroxidation was homogenized in phosphate buffer.

2.5 Statistical Analysis

all analysis was done using SPSS (version 16) and Microsoft Excel (2019) using one-way analysis of variance (ANOVA) and student T-test. Data were expressed as Mean± SEM with $p<0.05$ considered statistically significant

3. Results and Discussion

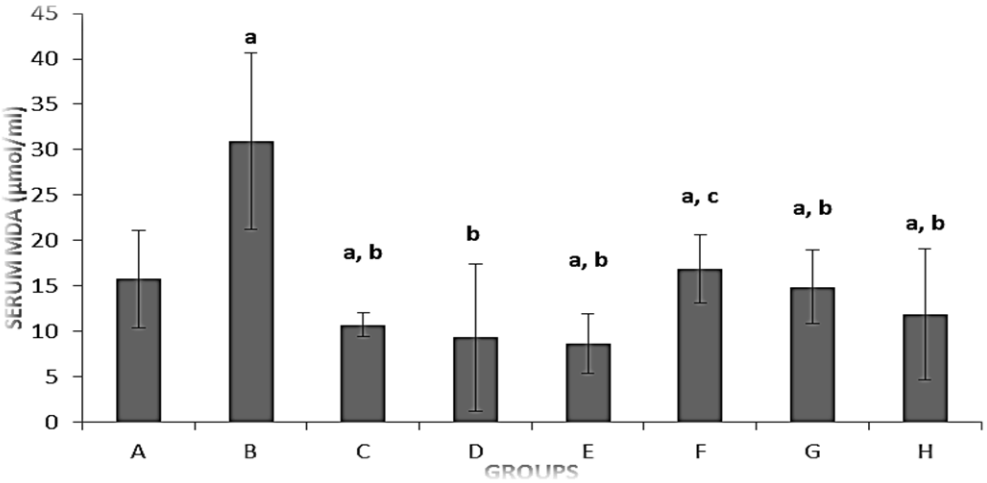
Table 1 illustrates how vitamin C protects adult male Wistar rats against alcohol induced dyslipidemia. Eight groups of rats were used in the study, and they each received a unique treatment. As the control group, group A only got distilled water, while group B received 6000 mg/kg of alcohol which induced dyslipidemia, while groups C to H received varying doses of vitamin C in addition to alcohol. The levels of cholesterol, triglyceride, HDL, and LDL were measured for each group. The results showed that alcohol administration led to an increase in cholesterol, triglyceride, and LDL levels, while HDL levels decreased. However, the co-administration of vitamin C with alcohol led to improvements in the lipid profile of the rats. Specifically, positive changes were noticed in the levels of lipid peroxidation, LDL, cholesterol, and triglyceride in all groups that received vitamin C. In addition, there was an improvement in HDL levels in groups C, D, and E. the findings imply that vitamin C may be helpful in preventing dyslipidemia and controlling alcohol induced toxicity.

Table 1. Protective effect of vitamin C against alcohol induced Dyslipidemia

Group	Treatment	Cholesterol (MMOL/L)	Triglyceride (MMOL/L)	HDL (MMOL/L)	LDL (MMOL/L)
A	Distilled water only	108.8±20.42	64±10.41	37.6±5.89	66.6±12.72
B	6000 mg/kg body weight of alcohol (30% v/v)	191.8±14.23	134±5.95^{A}	23.2±5.35	148.2±8.34
C	100 mg/kg of body weight of vitamin C	123.2±13.10^{B}	44±12.28	43.6±2.88 $^{A, B}$	46.2±19.61
D	200 mg/kg body weight of vitamin C	124±6.51^{B}	39.8±5.80^{A,B,C}	45.2±6.26^{A,B}	43.8±11.05^{B}
E	300 mg/kg body weight of vitamin C	100.6±5.85^{C,D}	35.2±16.75^{B}	49.4±5.50	39.4±6.02^{D}
F	6000 mg/kg body weight of alcohol and 100 mg/kg body weight of vitamin C	127.4±46.61	34.6±15.99^{B}	49.2±15.70^{B,E}	56.2±37.44^{A,B,C,E}
G	6000 mg/kg body weight of alcohol and 200 mg/kg body weight of vitamin C	116.8±12.11^{A,C,D,E,F}	65.6±52.60^{A,B,C}	41.4±10.40	48.4±9.93 $^{A, B, C, D, E, F}$
H	6000 mg/kg body weight of alcohol and 300 mg/kg body weight of vitamin	108.4±2.19^{A,C,D,E,F,G}	58.2±15.30^{D,G}	38±11.89	44.6±14.06$^{A, B, C, D, E, F}$

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 B, C- values were significant when compared to C, D- were significant when compared to D, e- values were significant when compared to E, F-values were significant when compared to F, G- values were significant when compared to G



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

A; distilled water only, B; 6000 mg/kg body weight of alcohol, 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 and 100 mg/kg body weight of vitamin C, G; 600 mg/kg body weight of alcohol and 200 mg/kg body weight of vitamin C, H; 6000 mg/kg of alcohol and 300 mg/kg body weight of vitamin C

Figure 1. Protective effect of vitamin C against alcohol induced increase in lipid peroxidation in adult male Wistar rats.

The graph in figure 1 shows the protective effect of vitamin C against alcohol-induced lipid peroxidation in adult male Wistar rats. The levels of lipid peroxidation are shown on the Y-axis, and the different treatment groups are shown on the X-axis. Group A, which received only distilled water, showed the lowest level of lipid peroxidation, while group B, which received alcohol, showed the highest level. Groups C to H received different doses of vitamin C either before or after alcohol administration. The findings show that vitamin Chas's preventive effect against alcohol induced dyslipidemia by lowering lipid peroxidation levels in proportion to vitamin C intake.

It has long been known that excessive levels of free radicals or reactive oxygen species (ROS) can cause immediate harm to lipid. Alcohol intake increases the formation of uncontrolled oxidative stress, which is the imbalance between pro-oxidant and antioxidant levels in favor of pro-oxidant [19]. According to Vanessa Fiorentino *et al.,* [20], dyslipidemia and increase in oxidative stress are major cause of cardiovascular disease including atherosclerosis and hypertension. Under oxidative stress, an increase in lipid peroxidation has an impact on cell membranes and other lipid containing cell structures the consumption of alcohol caused in increase in the level of lipid peroxidation signifying inflammations [21], also in groups administered with alcohol only pathological changes were seen in their lipid profile. Ethanol influence on lipid metabolism is complicated. When ethanol is present, it becomes the liver preferred fuel and replaces fats as a source of energy, which promotes fat storage [22]. The modified redox state caused by ethanol oxidation promoted lipogenesis by accelerating the synthesis of acylglycerols. Chronic alcohol consumption impairs mitochondrial oxidative capacity accelerating the development of the fatty liver [23]. Fats accumulation stimulates the secretion of lipoproteins and the development of hyperlipidemia. The rise in LDL, cholesterol and triglyceride is due to increased oxidative stress, which is a critical early event in the pathogenesis of cardiovascular disease such as atherosclerosis, previous studies has shown that high blood cholesterol, triglycerides low density lipoprotein are leading causes of cardiovascular diseases, also there is a complex relationship between alcohol consumption and the increased risk of cardiovascular diseases. As seen in our study results the metabolism of alcohol may lead to the increased production of dangerous lipid byproduct level, for example, LDL may negatively affect endothelial cell function directly by increasing the formation of free radicals that deactivate nitric oxides by building up in the intima region of increased endothelial permeability [24-30]. Furthermore, oxidized LDL causes an increase in monocyte concentration in lesions and stimulates the release of cytokines and growth factors, which in turn causes the smooth muscle to migrate and proliferate from the media into the intima. This process transforms a fatty streak into a mature fibrofatty atheroma and it aids in the progression of atherosclerotic lesions, the primary cause of cardiovascular diseases [31]. The consumption of alcohol leads to an increase in hepatic cholesterol ester levels and a rise in blood cholesterol. Moreover, ethanol alters the cholesterol domains in the plasma membrane, which affect the trans bilayer fluidity gradients and are linked to lower Ca^{2+}, Na^{+}, and K^{+}- ATPasa activity [32-33]. As a result of the generation of reactive oxygen species (ROS), brought on by the metabolism of alcohol, which will ultimately contribute to the dysregulation of physiological processes and a depletion in antioxidant vitamins and minerals, the HDL level in the alcohol group of rats decreased. Vitamin C has been utilized to alleviate oxidative stress in ischemia/reperfusion (I/R) processes in many pharmacological trials, and it is well known to exert pleiotropic therapeutic effects in a number of human pathologies, including cardiovascular disease, the result of our studies shows that in the co- administration groups there was positive changes in the lipid profile content of the blood and also reduction in the level of lipid peroxidation, this could be brought on by an increase in vitamin C levels in the blood, which will produce an ion-dependent pro-oxidant effect [34]. Vitamin C also functions as an antioxidant and scavenger of free radicals, which would reduce lipid peroxidation and prevent oxidative stress related damage to DNA, protein and lipids. Moreover, vitamin C can boost the function of other antioxidant like glutathione and vitamin E and replenish them, lowering oxidative stress even more [35]. Moreover, vitamin C can influence lipid metabolism by decreasing the liver production of lipids and lipoprotein, boosting the removal of lipids from circulation and improving insulin sensitivity, all of which can improve lipid metabolism, in our study higher dose of vitamin C showed high positive changes while low dose showed slight improvement [36].

4. Conclusion

Vitamin C supplementation was able to reduce alcohol induced increase lipid disorder and peroxidation, thereby averting development of atherosclerosis. Thus, Vitamin C may be used in the management of alcohol induced toxicity, since it had favorable effect of lipid profile and peroxidation

References

[1] M.C. Toffolo, A.S. de Aguiar-Nemer, V.A. da Silva-Fonseca, Alcohol: effects on nutritional status, lipid profile and blood pressure. *Journal of Endocrinology and Metabolism*. 2(6) (2012) 205-211. [DOI]

[2] G. Sebastiani, C. Borrás-Novell, M. Alsina Casanova, M. Pascual Tutusaus, S. Ferrero Martínez, M. D. Gómez Roig, O. García-Algar. The effects of alcohol and drugs of abuse on maternal nutritional profile during pregnancy. *Nutrients*, 10(8) (2018) 1008. [DOI] [PubMed]

[3] S. Singh, C. Brocker, V. Koppaka, Y. Chen, B.C. Jackson, A. Matsumoto, D.C. Thompson, V. Vasiliou, Aldehyde dehydrogenases in cellular responses to oxidative/electrophilicstress. *Free radical biology and medicine*, 56 (2013) 89-101. [DOI] [PubMed]

[4] B. Xi, S.P. Veeranki, M. Zhao, C. Ma, Y. Yan, J. Mi, Relationship of alcohol consumption to all-cause, cardiovascular, and cancer-related mortality in US adults. *Journal of the American College of Cardiology*, 70(8) (2017) 913-922. [DOI] [PubMed]

[5] J. Rehm, D. Baliunas, G.L. Borges, K. Graham, H. Irving, T. Kehoe, C.D. Parry, J. Patra, S. Popova, V. Poznyak, M. Roerecke, The relation between different dimensions of alcohol consumption and burden of disease: an overview. *Addiction,* 105(5) (2010) 817-843. [DOI] [PubMed]

[6] M.O. Afolabi, A.E. Ayilara, O.A. Akinyemi, O.J. Ola-Olorun, Survey of drug use among young people in Ife, Nigeria. *African Journal of Drug and Alcohol Studies*, 11(2) (2012).

[7] J. Rehm, S. Imtiaz, A narrative review of alcohol consumption as a risk factor for global burden of disease. *Substance abuse treatment, prevention, and policy*, 11(1) (2016)1-2. [DOI] [PubMed]

[8] J. Rehm, G.E. Gmel Sr, G. Gmel, O.S. Hasan, S. Imtiaz, S. Popova, C. Probst, M. Roerecke, R. Room, A.V. Samokhvalov, K.D. Shield, The relationship between different dimensions of alcohol use and the burden of disease—an update. *Addiction,* 112(6) (2017) 968-1001. [DOI] [PubMed]

[9] F.S. Al-Fartusie, S.N. Mohssan, Essential trace elements and their vital roles in human body. Indian Journal of Advances in Chemical Science, 5(3) (2017) 127-136.

[10] J.L. Barrita, M.D. Sánchez, Antioxidant role of ascorbic acid and his protective effects on chronic diseases. *Oxidative Stress and Chronic Degenerative Diseases - A Role for Antioxidants*, 449 (2013) 450-484.

[11] F.E. Pehlivan. Vitamin C: An antioxidant agent. *Vitamin C,* 2 (2017) 23-35. [DOI]

[12] A. Yadav, R. Kumari, A. Yadav, J.P. Mishra, S. Srivatva, S. Prabha, Antioxidants and its functions in human body-A Review. *Research in Environment and Life Sciences*, 9(11) (2016) 1328-1331.

[13] Sorice, E. Guerriero, F. Capone, G. Colonna, G. Castello, S. Costantini, Ascorbic acid: its role in immune system and chronic inflammation diseases. *Mini reviews in medicinal chemistry*, 14(5) (2014) 444-452. [DOI] [PubMed]

[14] M. G. Kashiouris, M. L'Heureux, C.A. Cable, B.J. Fisher, S.W. Leichtle, A.A. Fowler, The emerging role of vitamin C as a treatment for sepsis. *Nutrients*, 12(2) (2020) 292. [DOI] [PubMed]

[15] E. Gianazza, M. Brioschi, A. Martinez Fernandez, F. Casalnuovo, A. Altomare, G. Aldini, C. Banfi, Lipid peroxidation in atherosclerotic cardiovascular diseases. *Antioxidants & redox signaling*, 34(1) (2021) 49-98. [DOI] [PubMed]

[16] S.A. Vieira, G. Zhang, E.A. Decker, Biological implications of lipid oxidation products. *Journal of the American Oil Chemists' Society*, 94 (2017) 339-351. [DOI]

[17] National Research Council, (2011). InGuide for the Care and Use of Laboratory Animals. 8th edition. *National Academies Press,* United States.

[18] J.A. Buege, S.D. Aust. (1978). Methods in Enzymology Vol. *LII Biomembranes*.

[19] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative medicine and cellular longevity*, (2014). [DOI] [PubMed]

[20] T. Vanessa Fiorentino, A. Prioletta, P. Zuo, F. Folli, Hyperglycemia-induced oxidative stress and its role in diabetes mellitus related cardiovascular diseases. *Current pharmaceutical design*, 19(32) (2013) 5695-5703. [DOI] [PubMed]

[21] F. Ito, Y. Sono, T. Ito, Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: oxidative stress in diabetes, atherosclerosis, and chronic inflammation. *Antioxidants*, 8(3) (2019) 72. [DOI] [PubMed]

[22] C.S. Lieber, Alcohol-induced hepatotoxicity. *InHepatotoxicology CRC Press*, (2020). 481-523. [DOI]

[23] A. Engin, Non-alcoholic fatty liver disease. *Obesity and Lipotoxicity*, (2017) 443-467. [DOI]

[24] U. Wali, A.A. Binji, K. Ahmad, A. Usman. Lipid peroxidation and lipid profile in hypertensive patients in Sokoto, Nigeria. *Nigerian Journal of Basic and Applied Sciences*, 20(3) (2012)199-204.

[25] M.M. Kanter, P.M. Kris-Etherton, M.L. Fernandez, K.C. Vickers, D.L. Katz, Exploring the factors that affect blood cholesterol and heart disease risk: is dietary cholesterol as bad for you as history leads us to believe?. *Advances in Nutrition*, 3(5) (2012) 711-717. [DOI] [PubMed]

[26] B.G. Nordestgaard, A. Varbo, Triglycerides and cardiovascular disease. *The Lancet*, 384 (9943) (2014) 626-635. [DOI]

[27] J. Poss, F. Custodis, C. Werner, O. Weingartner, M. Bohm, U. Laufs, Cardiovascular disease and dyslipidemia: beyond LDL. *Current pharmaceutical design,* 17(9) (2011) 861-870. [DOI] [PubMed]
[28] Y. Jiang, T. Zhang, P. Kusumanchi, S. Han, Z. Yang, S. Liangpunsakul, Alcohol metabolizing enzymes, microsomal ethanol oxidizing system, cytochrome P450 2E1, catalase, and aldehyde dehydrogenase in alcohol-associated liver disease. *Biomedicines,* 8(3) (2020) 50. [DOI] [PubMed]
[29] M. Singh, A. Kapoor, A. Bhatnagar, Oxidative and reductive metabolism of lipid-peroxidation derived carbonyls. *Chemico-Biological Interactions*, 234 (2015) 261-73. [DOI] [PubMed]
[30] W.J. Lee, H.C. Ou, W.C. Hsu, M.M. Chou, J.J. Tseng, S.L. Hsu, K.L. Tsai, W.H. Sheu, Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation, and inflammation in human endothelial cells. *Journal of vascular surgery,* 52(5) (2010) 1290-1300. [DOI] [PubMed]
[31] B. Pamukcu, G.Y. Lip, A. Devitt, H. Griffiths, E. Shantsila, The role of monocytes in atherosclerotic coronary artery disease. *Annals of medicine*, 42(6) (2010) 394-403. [DOI] [PubMed]
[32] A.T Klop, M.C. do Rego, Cabezas, Alcohol and plasma triglycerides. *Current opinion in lipidology,* 24(4) (2013) 321-326. [DOI] [PubMed]
[33] E.A. Brinton, Effects of ethanol intake on lipoproteins. *Current atherosclerosis reports*, 14(2) (2012) 108-114. [DOI] [PubMed]
[34] M. Berretta, V. Quagliariello, N. Maurea, R. Di Francia, S. Sharifi, G. Facchini, L. Rinaldi, M. Piezzo, C. Manuela, G. Nunnari, M. Montopoli. Multiple effects of ascorbic acid against chronic diseases: Updated evidence from preclinical and clinical studies. *Antioxidants,* 9(12) (2020) 1182. [DOI] [PubMed]
[35] S.M. El-Bahr, Biochemistry of free radicals and oxidative stress. *Biochemistry*, 1 (5567/5cjiŋt) (2013) 11.
[36] V Amalan, N Vijayakumar, D Indumathi, A Ramakrishnan, Antidiabetic and antihyperlipidemic activity of p-coumaric acid in diabetic rats, role of pancreatic GLUT 2: In vivo approach. *Biomedicine & Pharmacotherapy*, 84 (2016) 230-236. [DOI] [PubMed]

Conflict of interest

The Authors have no conflicts of interest to declare that they are relevant to the content of this article.

Does the Article Screened for Similarity?

Yes.

Author Contribution Statement

OYESOLA, Olusoji Adebusoye-Conceptualization, methodology, resources, supervision; OWOEYE, Ifedolapo Ibukuoluwa Gloria-Writing, reviewing and editing;EHIREMEN, Samuel Ehimare-Methodology, resources;ADENEKAN, Sunday Oluwaseun-Methodology, resources; GEORGE, Emmanuel Taiwo-Conceptualization, methodology, formal analysis, writing (original draft). All the authors read and approved the final version of the manuscript.

Ethics Approval

This study was approved by Institutional Review Board.

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