Global Journal of Science Frontier Research: B
Chemistry
Volume 22 Issue 1 Version 1.0 Year 2022
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4626 & Print ISSN: 0975-5896

Cardiac Oxidative Status in CCl_4-Exposed Rats Treated with Extracts of *Dialium guineense* Stem Bark

By Abu O. D., Iyare H. E. & Ogboi K. U.

University of Benin

Abstract- The present study investigated cardiac oxidative status in carbon tetrachloride (CCl_4)-exposed rats treated with aqueous and ethanol extracts of *Dialium guineense* stem bark. Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg body weight, bwt. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated in heart homogenate.

Keywords: *cardioprotection, dialium guineense, heart, lipid peroxidation, oxidative stress.*

GJSFR-B Classification: *DDC Code: 338.4766288 LCC Code: HD9502.5.B543*

Strictly as per the compliance and regulations of:

Cardiac Oxidative Status in CCl_4-Exposed Rats Treated with Extracts of *Dialium guineense* Stem Bark

Abu O. D. [α], Iyare H. E. [σ] & Ogboi K. U. [ρ]

Abstract- The present study investigated cardiac oxidative status in carbon tetrachloride (CCl_4)-exposed rats treated with aqueous and ethanol extracts of *Dialium guineense* stem bark. Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg body weight, bwt. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days. Activities of antioxidant enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated in heart homogenate. The results showed that there were no significant differences in the concentrations of cardiac total protein (TP) among the groups ($p>0.05$). The activities of the antioxidant enzymes and level of reduced glutathione (GSH) were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract treatment ($p<0.05$). However, the level of cardiac malondialdehyde (MDA) increased by CCl_4 intoxication was significantly reduced after treatment ($p<0.05$). These results indicate that aqueous and ethanol extracts of *D. guineense* stem bark may enhance antioxidant defense in rats hearts exposed to CCl_4.

Keywords: cardioprotection, dialium guineense, heart, lipid peroxidation, oxidative stress.

I. Introduction

Carbon tetrachloride (CCl_4) is a colorless liquid with a "sweet" smell that can be detected at low levels [1]. Its production has steeply declined since the 1980s due to environmental concerns and the decreased demand for chlorofluorocarbons (CFCs), such as the Freons dichlorodifluoromethane (F-12) and trichlorofluro- methane (F-11), which are used primarily as refrigerants [2]. It is also used in petroleum refining, pharmaceutical manufacturing, as an industrial solvent, in the processing of fats, oils, and rubber, and in laboratory applications [3]. Currently, CCl_4 is not permitted in products intended for home use. The primary routes of potential human exposure to CCl_4 are inhalation, ingestion, and dermal contact. The general population is most likely to be exposed to CCl_4 through air and drinking water[4 – 6]. In humans and animals, CCl_4 is rapidly absorbed by any route of exposure. Once absorbed, it is widely distributed among tissues, especially those with high lipid content, reaching peak concentrations in <1– 6 h, depending on exposure concentration or dose. The compound is metabolized in the body, primarily by the liver, but also in the kidney, lung, and other tissues containing cytochrome P450 (CYP450). The fraction of the compound that is metabolized varies with dose [7, 8].

The heart is a muscular organ which pumps blood through the blood vessels of the circulatory system [9]. Blood provides the animal's body with oxygen and nutrients as well as assist in the removal of metabolic wastes. In humans, the heart is located between the lungs in the middle compartment of the chest [10 - 12]. The heart is effectively a syncytium, a meshwork of cardiac muscle cells interconnected by contiguous cytoplasmic bridges [13 - 15].

Plants are at the center of Traditional Medicine. Their use in disease management is as old as man [16, 17]. Medicinal plants serve as cheap alternative to orthodox medicine since they are readily available [18 - 20]. *Dialium guineense* is a medicinal plant used in folklore medicine for the treatment of infections such as diarrhea, severe cough, bronchitis, wound, stomachaches, malaria, jaundice, ulcer and hemorrhoids [21, 22]. At present not much is known about the potential of extracts of *D. guineense* stem bark to protect against CCl_4-induced cardio-toxicity in rats. The aim of this study was to investigate cardiac oxidative status in CCl_4-exposed rats treated with aqueous and ethanol extracts of *D. guineense* stem bark.

II. Materials and Methods

a) Chemicals

All chemicals and reagents used in this study were of analytical grade and they were products of Sigma-Aldrich Ltd. (USA).

Author α ρ: Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
e-mail: osahon.abu@uniben.edu
Author σ: Department of Science Laboratory Technology, Shaka Polytechnic, Benin City, Nigeria.

b) Collection of Plant Material

The stem barks of *D. guineense* were obtained from Auchi, Edo State, Nigeria and authenticated at the herbarium of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria (No. UBH_D330).

c) Plant Preparation and Extraction

The stem bark was washed and shade-dried at room temperature for a period of two weeks and crushed into small pieces using clean mortar and pestle. Aqueous and ethanol extracts of the stem bark were obtained using cold maceration method as described previously [23].

d) Experimental Rats

Adult male Wistar rats (n = 25) weighing 170 – 190 g (mean weight = 180 ± 10 g) were obtained from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats were housed in metal cages under standard laboratory conditions: room temperature, 55 – 65% humidity and 12-h light/12-h dark cycle. They were allowed free access to rat feed (pelletized growers mash) and clean drinking water. Prior to commencement of the study, the rats were acclimatized to the laboratory environment for one week. The study protocol was approved by the University of Benin Faculty of Life Sciences Ethical Committee on Animal Use.

e) Experimental Design

The rats were randomly assigned to five groups (5 rats per group): normal control, CCl_4 control, silymarin, aqueous extract and ethanol extract groups. With the exception of normal control, the rats were exposed to CCl_4 at a single oral dose of 1.0 mL/kg bwt [23]. Rats in the silymarin group were administered silymarin (standard cardioprotective drug) at a dose of 100 mg/kg bwt, while those in the two treatment groups received 1000 mg/kg bwt of aqueous or ethanol extract orally for 28 days.

f) Tissue Sample Collection and Preparation

At the end of the treatment period, the rats were euthanized and their hearts excised, and used to prepare 20% tissue homogenate. The homogenate was centrifuged at 2000 rpm for 10 min to obtain supernatant which was used for biochemical analysis.

g) Biochemical Analyses

The activities of catalase, SOD and GPx were determined [24 - 26]. Levels of total protein, MDA and GSH were also measured [27 - 29]. The activity of GR was determined using a previously described method [30].

III. Results

a) Effect of Extracts of D. guineense Stem Bark on Relative Organ Weight

As shown in Table 1, there were no significant differences in relative organ weight among the groups ($p > 0.05$).

Table 1: Relative Organ Weights of Rats

Group	Relative organ weight x 10^{-2}
Normal Control	3.34± 0.54
CCl_4 Control	3.02± 0.10
Silymarin	3.17± 0.16
Aqueous Extract	3.52± 0.24
Ethanol Extract	2.96± 0.14

Data are relative organ weights and are expressed as mean ± SEM (n = 5).

b) Effect of Extracts of D. guineense Stem Bark on Oxidative Status in Rat Heart

There were no significant differences in the concentrations of cardiac TP among the groups ($p>0.05$). The activities of the antioxidant enzymes and level of GSH were significantly lower in CCl_4 control group than in normal control group, but they were increased by extract treatment ($p<0.05$). However, the level of cardiac MDA increased by CCl_4 intoxication was significantly reduced after treatment ($p<0.05$). These results are shown in Figures 1 to 3.

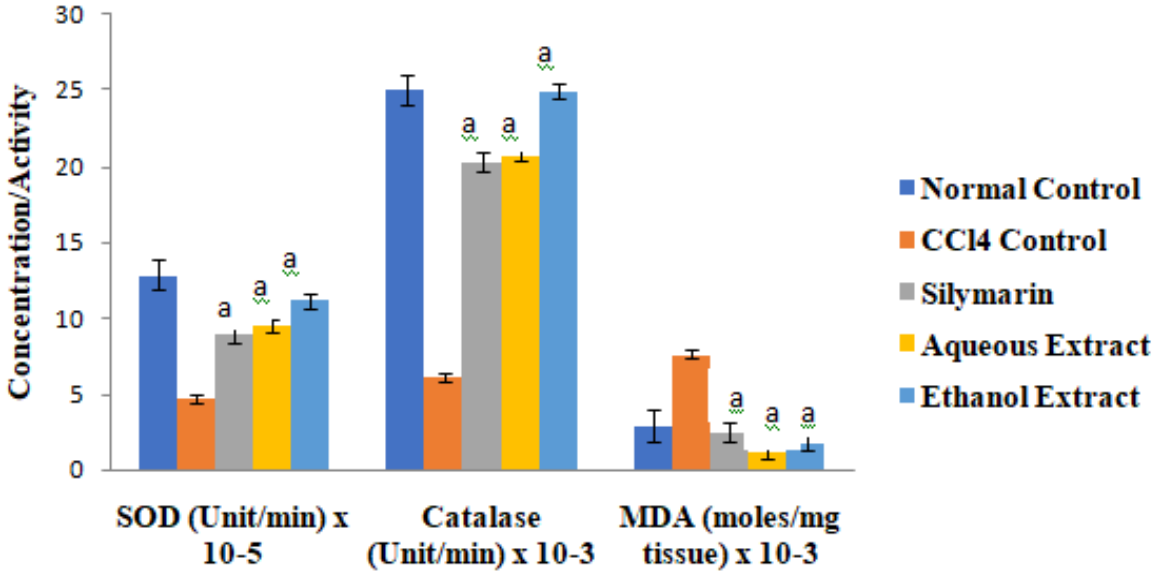

Figure 1: Effect of Extracts of *D. guineense* Stem Bark on Markers of Oxidative Stress in Rat Heart

Data are oxidative stress markers, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

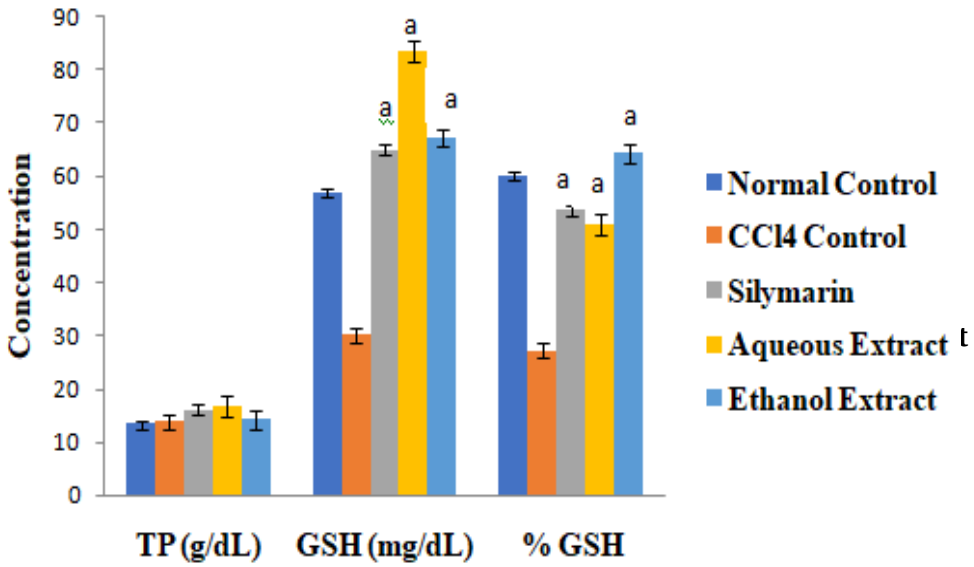

Figure 2: Effect of Extracts of *D. guineense* Stem Bark on Some Oxidative Stress Parameters

Data are oxidative stress markers, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

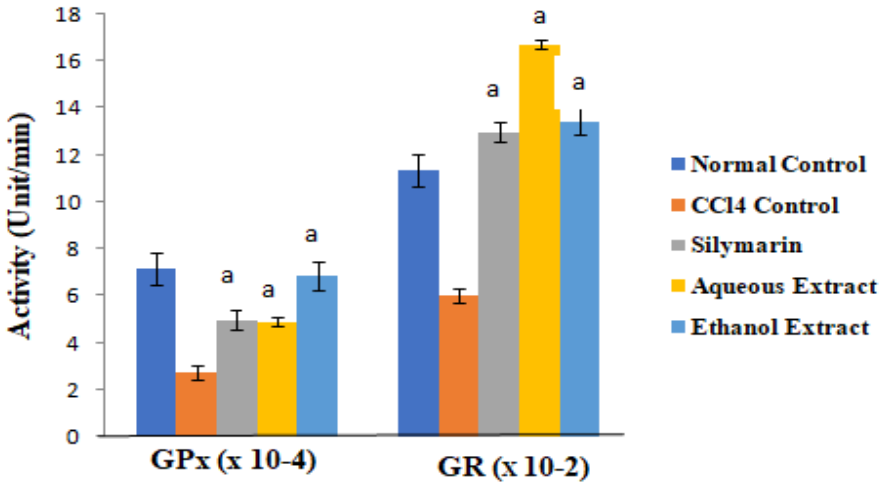

Figure 3: Effect of Extracts of *D. guineense* Stem Bark on Rat Oxidative Status

Data are oxidative stress parameters, and are expressed as mean ± SEM. $^{a}p < 0.05$, when compared with CCl_4 control group.

IV. Discussion

In animals, CCl_4 is rapidly absorbed via any route of exposure. Once absorbed, it is widely distributed among tissues, especially those with high lipid content. It is metabolized in the body, primarily by the liver, but also in the kidney, lung, and other tissues containing CYP450.The poison reaches its maximum concentration in the liver within 3 h of administration, thereafter it falls and by 24 h it is completely cleared from the organ [7, 8].

Tissue injury produced by CCl_4 is mediated by two major processes resulting from bioactivation in the endoplasmic reticulum (ER) and mitochondria of centrilobular hepatocytes [31]; haloalkylation of cellular macromolecules by reactive metabolites such as trichloromethyl free radical or trichloromethyl peroxyl free radical [32–34]; and lipid peroxidation [35].

Reactive oxygen species (ROS) and oxidative stress have been shown to play an important role in the etiopathogenesis of tissue injury. The role of oxidative stress in cardiac hypertrophy and remodeling has been demonstrated. An increased generation of ROS in the vascular wall and a reduction of nitric oxide (NO) bioavailability lead to endothelial dysfunction in atherogenesis [36, 37]. The ROS cause damage to cellular structures within the vascular wall, thereby triggering several redox-sensitive transcriptional pathways, shifting the cell towards a proatherogenic transcriptomic profile. Animal models of atherosclerosis demonstrate the involvement of ROS in atherosclerosis by the accumulation of lipid peroxidation products and induction of inflammatory genes and activation of matrix metalloproteinases [38, 39]. The ROS and reactive nitrogen species (RNS) produced by the endothelium promote oxidative modification of low-density lipoprotein-cholesterol (LDL-C) in the phase that precedes the transfer into the subendothelial space of the arterial wall, where they initiate atherosclerosis [40].

This study investigated cardiac oxidative status in CCl_4-exposed rats treated with extracts of *Dialium guineense* stem bark. The results showed that the activities of the antioxidant enzymes measured as well as level of GSH were significantly lower in CCl_4 control group than in normal control group, but these parameters were increased by extract treatment. However, the level of cardiac MDA increased by CCl_4 was significantly reduced after treatment. These results suggest that extracts of *D. guineense* stem bark may enhance antioxidant defense in rat heart exposed to CCl_4. The capacity of extracts of the medicinal plant to potentiate natural antioxidant defense system has been reported [41-43]. Plants rich in polyphenols are reported to possess good antioxidant capacity [44-46]. Plants with cardioprotective potential have been shown to contain a variety of bioactive compounds, such as diosgenin, isoflavones, sulforaphane, carotenoids, catechins, quercetin, allicin, cardiac glycosides, saponin-shatavarins 1-1V, cyclovirobuxine D and triterpenes/triterpenoids [47–49].

The cardioprotective effect of medicinal plants may involve attenuation of the damage in cardiac muscle cells, vascular smooth muscle cells (VSMCs), endothelial cells (ECs), and macrophages and monocytes. In cardiomyocytes, cardioprotective agents may promote the opening of K_{ATP} channel, increased secretion of atrial natriuretic peptide, as well as the regulation of cardiac hypertrophy, oxidative stress, and apoptosis [50, 51].

V. Conclusion

The results of this study suggest that aqueous and ethanol stem bark extracts of *D. guineense* enhance

antioxidant defense in rat heart exposed to CCl_4. Their bioactive molecules may exert cardioprotective function via suppression of specific factors, regulation of key enzymes, and scavenging of oxygen-free radicals.

References Références Referencias

1. Doherty, R.E. (2000). "A History of the production and Uses of Carbon Tetrachloride, Tetrachloroethylene, Trichloroethylene and 1,1,1-Trichloroethane in the United States". *Environmental Forensics*1(1): 69-81.
2. Doherty, A.T. (1996). An investigation into the activation and deactivation of chlorinated alkanes. *J Phys Chem.* 94: 3277–3283.
3. IARC (1999). Carbon tetrachloride. In *Re-evaluation of Some Organic Chemicals, Hydrazine, and Hydrogen Peroxide.* IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans. *International Agency for Research on Cancer.* 71: 401-432.
4. Rossberg. M., Wilhelm, L., Gerhard, P., Adolf, T., Eberhard-Ludwig, D., Ernst, L., Heinz, J., Peter, K., Heinz, S., Richard, C., Uwe, B., Karl-August, L., Theodore, R.T., Eckhard, L. and Klaus, K.B., "Chlorinated Hydrocarbons" in Ullmann's Encyclopedia of Industrial Chemistry, 2006 Wiley-VCH, Weinheim.
5. Reusch. W. (2013)". Introduction to Nuclear Magnetic Resonance Spectroscopy". *Virtual Textbook of Organic Chemistry*. Michigan State University.
6. Holbrook, M.T. (1993). Carbon tetrachloride. In: Kroschwitz, JI; Howe-Grant, M; eds. Kirk-Othmer encyclopedia of chemical technology. 4th edition. Vol. 5. New York, NY: John Wiley and Sons. Pp. 1062–1072.
7. Reinke, L.A. and Janzen, E.G. (1991). Detection of spin adducts in blood after administration of carbon tetrachloride to rats. *Chem. Biol. Interact.* 78: 155 – 165.
8. Sanzgiri, U.Y., Kim, H.J. and Muralidhara, S. (1995). Effect of route and pattern of exposure on the pharmacokinetics and acute hepatotoxicity of carbon tetrachloride. *Toxicol Appl Pharmacol.* 134: 148–154.
9. Benson, J.M., Tibbetts, B.M. and Thrall, K.D. (2001). Uptake, tissue distribution, and fate of inhaled carbon tetrachloride: comparison of rat, mouse, and hamster. *Inhal Toxicol.* 13: 207 – 217.
10. Taber, C.W. and Venes, D. (2009). Taber's cyclopedic medical dictionary. Davies F.A. Co. Pp. 1018 – 1023.
11. Moore, K.L., Dalley, A.F. and Agur, A.M.R. (2009). Clinically oriented anatomy. Wolters Kluwel Health/Lippincott William & Wilkins. Pp. 127 – 173.
12. Guyton, A.C. and Hall, J.E. (2006). *Textbook of Medical Physiology* (11th ed.). Philadelphia: Elsevier Saunder. Pp. 24.
13. MacDonald, M. (2009). *Your Body: The Missing Manual*. Sebastopol, CA: Pogue Press. Pp.40.
14. Maton, A., Jean, H., Charles, W.M., Susan, J., Maryanna, Q.W., David, L., Jill, and Wright, D. (1993). *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall.
15. DuBose, T.J. (1996). *Fetal Sonography*, pp. 263–274; Philadelphia: WB Saunders Fennema, O. (2008). *Fennema's Food Chemistry*. CRC Press Taylor & Francis. Pp. 454–455.
16. Brattin, W.J., Glende, E.A. and Recknagel, R.O. (1985). Pathological mechanisms in carbon tetracholoride hepatotoxicity. *J Free Radic Biol Med*. 1 (1): 27 - 38.
17. Grabley, T. and Akuodor, C.W. (2010). Medicinal Plants and Traditional Medicine. Watermelon Crop Information. *Cucurbit Breeding Horticultural Science.*
18. Abu, O.D. and Onoagbe, I.O. (2019). Biochemical effect of aqueous extract *of Dialium Guineense* stem bark on oxidative status of normal Wistar rats. *International Journal of Clinical Biology and Biochemistry*. 1 (2): 15 – 18.
19. Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. *2nd Edition. Spectrum Books Ltd*., Ibadan, Nigeria. Pp. 289.
20. Akah, P.A. and Nwabie, A.I. (1994). Evaluation of Nigerian traditional medicinal plants used for rheumatic inflammatory disorders. *Journal of Ethnopharmarcology,* 42: 179 - 182.
21. Dalziel, J.M. and Hutchison, J. (1973). Flora of West Tropical Africa. Vol.1 (2nd Ed). The White friars Press Ltd. London. Pp. 561.
22. Bero, J., Ganfon, H., Jonville, M.C., Frederich, M., Gbaguidi, F., De, M.P., Moudachirou, M. and Quetin, L.J. (2009). *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. *Journal of Ethnopharmacology*. 122 (3): 439 - 444.
23. Abu, O.D., Imafidon, K.E. and Iribhogbe, M.E. (2017). Aqueous leaf extract of *Icacina trichanta* Oliv. ameliorates CCl_4-induced liver toxicity in Wistar rats. *Journal of the Nigerian Society of Experimental Biology.* 17 (3): 107 - 111.
24. Cohen, G., Dembie, C.D. and Marcus, J. (1970). Measurement of catalase activity in tissue extracts. Analytic *Biochemistry*. 34: 30 - 38.
25. Misra, H.R. and Fridovich, I. (1972). The role of superoxide anions in the auto oxidation of epinephrine and a single assay for superoxide dismutase. *J Biol. Chem*. 247: 3170 - 3175.
26. Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G. and Hockstra, W.G. (1973). Selenium biochemical role as a component of glutathione peroxidase. *Science*. 179: 588 - 590.

27. Henry, R.J., Sobel, C. and Beckman, S. (1957). Determination of serum protein by the Biuret reaction. *Anal. Chem*. 92 (149): 1 - 5.
28. Ellman, G.L. (1959). Tissue sulphydryl groups. *Archive of Biochemistry and Biophysics*. 82 (1): 70 – 77.
29. Guttridge, J.M.C. and Wilkins, C. (1982). Cancer dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. *FEBS Lett*. 137: 327 - 340.
30. Abu, O.D. and Ikponmwosa-Eweka, O. (2022). Evaluation of the Potential of Total saponins and Tannins of *Dialium guineense* Stem Bark in the Amelioration of Carbon Tetrachloride-Induced Renal Oxidative Stress. *SAU Science-Tech. Journal*. 7 (1): 42 - 50.
31. Raucy, J.L., Kraner, J.C., and Lasker, J.M. (1993). Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit Rev Toxicol.* 23: 1–20.
32. Mico, B.A. and Pohl, L.R. (1983). Reductive oxygenation of carbon tetrachloride. Trichloro-methylperoxyl radical as a possible intermediate in the conversion of carbon tetrachloride to electrophilic chlorine. *Arch Biochem Biophys.* 225: 596–609.
33. Poyer, J.L., McCay, P.B., and Lai, E.K. (1980). Confirmation of assignment of the trichloromethyl radical spin adduct detected by spin trapping during 13C-carbon tetrachloride metabolism in vitro and in vivo. Biochem *Biophys Res Commun.* 94:1154–1160.
34. Slater, T.F. (1981). Free radicals as reactive intermediates in tissue injury. Adv Exp Med Biol. 136: 575–589.
35. Weber, L.W., Boll, M., and Stampfl, A. (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol.* 33: 105–136.
36. Lee, R., Margaritis, M., Channon, K.M. and Antoniades, C. (2012). "Evaluating oxidative stress in human cardiovascular disease: methodological aspects and considerations," *Current Medicinal Chemistry*. 19 (16): 2504 – 2520.
37. Channon, K.M. and Guzik, T.J. (2002). "Mechanisms of superoxide production in human blood vessels: relationship to endothelial dysfunction, clinical and genetic risk factors," *Journal of Physiology and Pharmacology.* 53: 515–524.
38. Liao, F., Andalibi, A., Qiao, J.H., Allayee, H., Fogelman, A.M. and Lusis, A.J. (1994). "Genetic evidence for a common pathway mediating oxidative stress, inflammatory gene induction, and aortic fatty streak formation in mice," *Journal of Clinical Investigation*. 94: 877–884.
39. Rajagopalan, S., Meng, X.P. Ramasamy, S., Harrison, D.G. and Galis, Z.S. (1996). "Reactive oxygen species produced by macrophage-derived foam cells regulate the activity of vascular matrix metalloproteinases in vitro: implications for atherosclerotic plaque stability," *Journal of Clinical Investigation*. 98 (11): 2572– 2579.
40. Stocker, R. and Keaney, J.F. (2004). "Role of oxidative modifications in atherosclerosis," *Physiological Reviews*. 84 (4): 1381–1478.
41. Abu, O.D., Imafidon, K.E. and Obayuwana, H.O. (2020). Evaluation of *in vitro* antioxidant activities of extracts of *Citrullus lanatus* seed. *Global Scientific Journal*. 8 (10): 1049 – 1060.
42. Abu, O.D., Imafidon, K.E., Obayuwana, H.O. and Onodje, S. (2020). Quantitative phytochemical evaluation and phenolic contents of extracts of *Citrullus lanatus* seed. *Int J Bioorg Chem Mol Biol.* 7 (1): 31 - 35.
43. Abu, O.D., Imafidon, K.E. and Obayuwana, H.O. (2021). Nephrotoxic and *in vivo* antioxidant effects of *citrulluslanatus* seed extract. *Biomedical Journal of Science and Technical Research.* 33 (5): 26281 – 26286.
44. Abu, O.D., Onoagbe, I.O. and Obahiagbon, O. (2020). *In Vitro* Antioxidant Activities of Isolated Total Saponins and Tannins of *Dialium Guineense* Stem Bark. *IAR Journal of Medical Sciences*. 1 (4): 193 – 199.
45. Abu, O.D., Onoagbe, I.O. and Obahiagbon, O. (2020). Phenolic contents of extracts of *Dialium guineense* stem bark. *American Journal of Sciences and Engineering Research.* 3 (4): 92– 96.
46. Shi, L.S., Liao, Y.R. and Su, M..J. (2010). Cardiac glycosides from *Antiaris toxicaria* with potent cardiotonic activity. *J Nat Prod*. 73 (7): 1214 – 1222.
47. Bopana, N. and Saxena, S. (2007). *Asparagus racemosus*—ethnopharmacological evaluation and conservation needs. *J Ethnopharmacol*. 110 (1): 1 – 15.
48. Sheena, N., Lakshmi, B. and Janardhanan, K. (2005). Therapeutic potential of *Ganoderma lucidum* (Fr.) P. Karst. *Nat Prod Rad*. 4: 382–386.
49. Jain, G., Jhalani, S., Agarwal, S. and Jain, K. (2007). Hypolipidemic and antiatherosclerotic effect of *Leptadenia pyrotechnica* extract in cholesterol fed rabbits. *Asian J Exp Sci*. 21 (1): 115–122.
50. Zanwar, A., Hegde, M. and Bodhankar, S. (2011). Cardioprotective activity of flax lignan concentrate extracted from seeds of *Linum usitatissimum* in isoprenalin induced myocardial necrosis in rats. *Interdiscip Toxicol*. 4 (2): 90 – 97.
51. Huang, C.L.Y. (2016). Chinese herbal medicine on cardiovascular diseases and the mechanisms of action. *Front Pharmacol*. 7: 469.