Annals of Science and Technology - A, Vol 8 (1): 59-64, 2023
Copyright: An Official *Journal* of the Nigerian Young Academy
ISSN: 2 544 6320

sciendo

ARTICLE

Effects of Butylated HydroxylToluene and Vitamin E on Cadmium-Lead toxicity on the liver of rats.

Helen E. Kadiri[1]*, Blessing Idholo[1]

[1]Department of Biochemistry, Delta State University, Abraka, Delta State, Nigeria.

Received 12th January, 2023, Accepted 4th April, 2023
DOI: 10.2478/ast-2023-0009

**Corresponding author*
Helen E. Kadiri1 (Ph.D) E-mail: hekad@yahoo.com.

Tel: +2348060465365

ABSTRACT
This study determined the effect of lead-cadmium toxicity in the liver of albino rats. 56 rats were divided into 7 groups of 8 rats each and were treated as follows : Group A Control (1 ml of diluent (corn oil); Group B (15mg/kg BW cadmium) ; Group C(50mg/kg BW lead); Group D (15mg/kg BW Cadmium and 50mg/kg BW lead); Group E (15mg/kg BW cadmium , 50mg/kg BW lead and 23mg/kg BW vitamin E);Group F (15mg/kg BW cadmium, 50mg/kg BW lead and 25mg/kg BW Butylated HydroxylToluene) and Group G (15 mg/kg BW Cd+50mg/kg BW lead+25mg/kg BW (BHT + vitamin E). Rats were sacrificed on the 29th and 56th day in sub-acute and chronic exposure respectively. Liver function, oxidative stress markers and histological examination were carried out. Results indicate that rats exposed to cadmium and lead suffered liver damage due to the significant ($P<0.05$) increase in Alanine Transferase, Aspartate transferase and Alkaline phosphatase, and significant decrease ($P<0.05$) in Total Protein and Albumin in the serum. Malonaldehyde (MDA) levels were also significantly increased ($P<0.05$) with a concomitant decrease in Superoxide dismutase, catalase and Glutathione peroxidase activities in both the chronic and acute exposure phase. Vitamin E and BHT singly had little ameliorative effect on the liver; however, a combination of these antioxidants reduced the enzymes levels significantly. Treatment with the antioxidants (Vitimin E and BHT) both singly and combined was also able to ameliorate the effects of the oxidative stress induced by the metals as was further confirmed by the histopathological reports.

Keywords: BHT; Vitamin E; Cadmium; Lead

BY-NC-ND © *Kadiri and Idholo* This work is licensed under the **Creative Commons Attribution-Non-Commercial-NoDerivs License 4.0**

Introduction

Metals are known to exist effortlessly on the earth's surface, and their availability in our surroundings can be very different between countries resulting in different concentrations of these heavy metals in different parts of the earth. Heavy metals distribution in the environment is determined by the characteristics of these metals and their effects on environmental factors (Khlif and Hamza-Chaffai, 2010). There are 92 naturally existing elements, roughly 30 metals and metalloids that are possibly lethal to humans. Contamination of the environment by heavy metals is either by natural or anthropogenic means. Ways of contamination include: weathering of the earth's crust and rocks, mining of mineral resources, erosion, industrial release, urban overspill, sewage waste, pest or disease control chemicals used on plants, air pollution fallout (Kadiri and Ekayoda, 2023). Cadmium (Cd) is an example of a heavy metal, it does not have a function in a biological system and is very toxic (Kadiri, and Ekayoda, 2019). Exposure to cadmium has been as a result of its presence in the environment due to activities of humans (Rahimzadeh *et al*, 2017); the use of corrosive chemicals, stabilizer in Poly-vinyl-chloride production and color pigments all result in the increase of cadmium pollution (Akporido and Kadiri, 2014; Genchi *et al*, 2020).

Lead (Pb), is an environmental genotoxic metal. It is ubiquitous and is found in many industrial materials such as batteries, cosmetics and pesticides. This has led to many countries enacting laws and standards to protect individuals from excess exposure to lead. Humans absorb lead through the skin, the alimentary and respiratory tract (Kumar *et al,* 2020). Studies indicate that lead alters lactate transport as well as glycolysis and their induced damage affects nucleic acids and many proteins of importance (Meramat *et al,* 2017).

Antioxidants can either be synthetic or natural; they are present in small amounts when compared to biological molecules that they defend. (Okoro and Kadiri, 2019; Jonscher *et al*, 2021). The mechanism of antioxidant defense is by inhibiting the destructive effects of free radicals. They are categorized as follows: endogens (glutathione, co-enzyme Q, etc.), which are produced by the cell; exogens, antioxidants gotten by the organism majorly through diet or through supplements with antioxidant formulations, and co-factors (copper, zinc, manganese, iron, and selenium). The consumption of antioxidant exogens can upsurge defense of the body and aid antioxidant endogens in fighting diseases. (Lorenzo *et al*, 2020). BHT is a man-made antioxidant used in the conservation of fatty foods as well as beauty products, where it hunts free radicals that causes lipid oxidation thereby stopping the deterioration process (Fasihnia et al,2020; Ousji and Sleno, 2020). Vitamin E is an essential constituent of our diet and it is known as the most operative lipid-soluble antioxidant found in the human system. It is composed of various subfamilies of which tocopherols and tocotrienols are the most studied.

Experimental

Experimental design

56 rats were purchased from the university farm in Abraka, Delta State, weighing between 100 to 150 g. The rats acclimatized for 14 days; they were divided into 7 groups of 8 rats per group. The groups are as follows; Group A Control 1 ml of diluent (corn oil); Group B (15 mg/kg cadmium); Group C (50 mg/kg lead); Group D (15 mg/kg cadmium and 50 mg/kg lead); Group E (15 mg/kg cadmium, 50 mg / kg lead and 23 mg / kg vitamin E); Group F (15mg /kg cadmium, 50 mg/kg Lead and 25 mg/kg BHT) and Group G [(15 mg /kg Cd + 50 mg / kg lead+ 25 mg / kg (BHT + vitamin E)]. Treatment of the rats was done orally for twenty-eight (28) days for sub-acute exposure, and four rats from each group were sacrificed on the 29th day, treatment continued to the 56th day for sub chronic, remaining four rats in each group was sacrificed. The experiment was conducted in compliance with the "Guide for care and use of laboratory animals" of the Faculty of Science, Delta State University, Abraka, Delta State (Approval no: REC/FOS/21/03).

SOURCES OF THE BHT, LEAD, CADMIUM AND VIT E

Butylated HydroxylToluene (C15H24O) was used in form of Sigma B1378, E321, and was dissolved in corn oil. The lead used was in form of lead tioxonitrate (Sigma). Cadmium used was in form of cadmium chloride (Sigma). Vitamin E used was in form of alpha-tocopherol acetate (Merck).

Estimation of Biochemical parameters:

The standard biochemical parameters determined in the serum were alanine aminotransferase (ALT). Aspartate aminotransferase (AST) and alkaline phosphatase (ALP), albumin and total protein were determined colorimetrically according to standard procedures using commercially available diagnostic kits (Randox Laboratories Limited, England).

Estimation of liver Pro- and antioxidant parameters

The antioxidant parameters estimated in the liver are Superoxide dismutase (SOD) (Misra and Fridovich, 1972), Catalase (CAT) (Kaplan et al, 1972), Malondialdehyde (MDA)(Buege, and Aust, 1968) , Glutathione peroxidase (GPx)(Ellman, 1959) and Glutathione-S-transferase (GST) (Ellman,1974).

Histological Studies

The tissues were fixed in 10% formyl saline for 48 hours and then embedded in paraffin. The tissues were cut into pieces of about 3 mm thick in pre-labelled tissue cassette. Serial sections of 5 micrometres thick were gotten from a hard block of tissue and stained using haematoxylin and eosin stains, after which they were passed through a combination of equivalent concentrations of xylene and alcohol. After clearance, the tissues were oven-dried and photomicrographs were taken with the aid of a JVC coloured video digital camera (JVC, China) which were mounted on an Olympus light microscope (Olympus UK Limited, United Kingdom).

Statistical Analysis

All results were denoted as means ±SD and all data were analyzed using Analysis of Variance (ANOVA). Significant differences between means were determined at ($P < 0.05$) confidence level using Posthoc test (least significant difference (LSD).

Results and Discussion

Humans are exposed to toxicants such as heavy metals (HM) on a daily basis in different doses and in a short term (sub-acute) or long term (sub chronic), HM toxicity can be as a result of anthropogenic or natural sources, also exposure to HM can be as a result of food sources and lifestyle or occupational hazards, The effects of cadmium-lead toxicity on serum AST, ALT, ALP ALB and Total Protein in rats treated with antioxidants Butylated hydroxyl toluene (BHT) and Vitamin E is shown in Table 1.The findings of this study demonstrated that rats exposed to Lead (Pb) and Cadmium (Cd), singly and collective, suffered severe liver damage which was detected by an increase in the serum levels of AST,

Table1: The effects of cadmium-lead toxicity on serum AST, ALT, ALP ALB and Total Protein in rats treated with antioxidants BHT and Vit.E.

Groups	**AST (u/l)**	**ALT (u/l)**	**ALP (u/l)**	**Alb.(u/l)**	**T. P (u/l)**
A (Control)	130.12 ± 4.09	90.12 ± 3.06	520.13 ± 6.96	10.16 ± 1.60	16.33±3.32
Sub-acute					
B (Cd)	175.00 ± 16.32^{a}	143.25 ± 10.05^{a}	562.19 ± 20.69^{b}	4.45 ± 1.61^{a}	9.50 ± 3.30^{a}
C (Pb)	170.00 ± 16.32^{a}	131.25 ± 8.37^{a}	555.10 ± 16.74^{b}	4.37 ± 3.43^{a}	10.30 ± 3.21^{a}
D (Cd +Pb)	178.87 ± 8.18^{a}	139.00 ± 19.25^{a}	570.10 ± 19.13^{c}	3.51 ± 1.35^{a}	8.02 ± 2.47^{a}
E (Cd+Pb+Vit E)	158.21 ± 16.36^{abc}	100.32 ± 16.04^{bc}	523.26 ± 16.28^{bc}	9.19 ± 4.02^{bc}	14.27 ± 3.30^{bc}
F (Cd+Pb+BHT)	151.45 ± 16.30^{abc}	101.35 ± 16.60^{bc}	534.50 ± 16.37^{bc}	8.92 ± 3.59^{bc}	14.63 ± 4.74^{bc}
G (Cd+Pb+Vit E+BHT)	143.18 ± 16.39^{bc}	96.88 ± 16.36^{bc}	523.35 ± 16.38^{bc}	9.52 ± 3.34^{bc}	15.60 ± 3.18^{bc}
Chronic					
B (Cd)	184.25 ± 3.88^{a}	174.00 ± 2.94^{a}	573.00 ± 2.22^{g}	6.26 ± 2.38^{a}	12.24 ± 2.21^{a}
C (Pb)	178.00 ± 7.48^{a}	172.62 ± 6.65^{a}	567.95 ± 1.48^{c}	8.32 ± 2.50^{a}	14.20 ± 2.87^{a}
D (Cd +Pb)	183.25 ± 7.85^{a}	180.00 ± 12.75^{a}	600.18 ± 30.84^{h}	5.10 ± 2.13^{a}	11.15 ± 2.94^{a}
E (Cd+Pb+Vit E)	157.00 ± 2.16^{bc}	140.00 ± 35.59^{abc}	582.12 ± 1.43^{h,j}	11.24 ± 4.50^{a}	15.40 ± 7.76^{bc}
F (Cd+Pb+BHT)	168.87 ± 4.2^{abc}	130.12 ± 42.97^{abc}	590.37 ± 8.17^{h}	10.19 ± 3.57^{a}	14.79 ± 6.63^{bc}
G (Cd+Pb+Vit E+BHT)	142.17 ± 1.40^{bc}	122.75 ± 24.78^{bc}	566.12 ± 4.40^{g}	13.23 ± 2.39^{a,b}	15.21 ± 4.63^{bc}

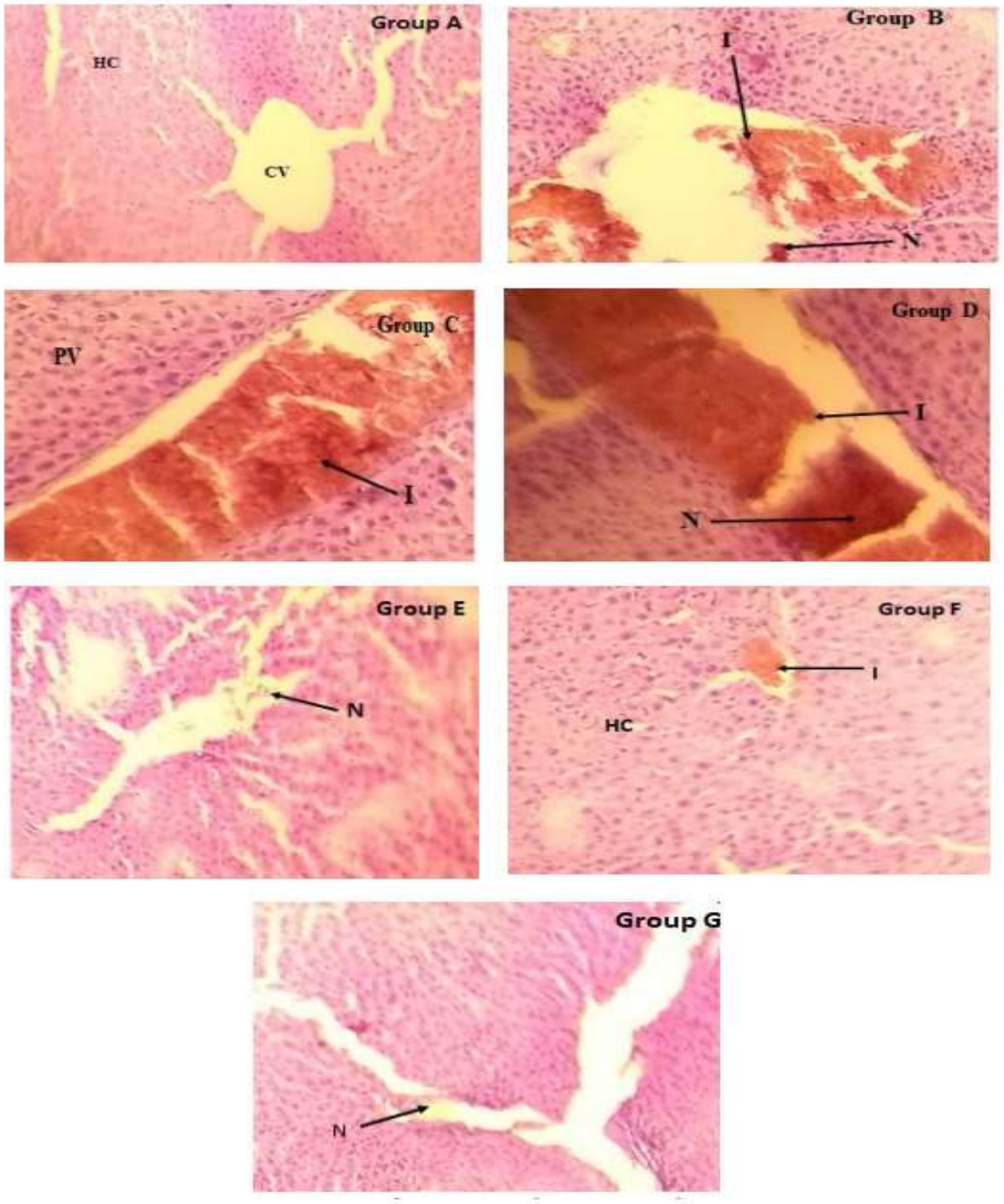
Values are represented in mean± SD. n=4.Startistical significance:a(P<0.05-0.001) from Normal, b(P<0.05-0.001) from Cd or Pb only, c(P<0.05-0.001) from Pb+Cd ,

ALT, and ALP and the upsurge in the activities of these enzymes compared to the control. Damage to liver cells changes their functional integrity, and leads to the leakage of enzymes into extracellular space (Chalasani et al, 2018; Kadiri and Ekayode, 2023). Exposure of albino rats to Pb or Cd or its combination causes liver damage, this assertion is supported by El-Sokkary et al. (2010), and Kadiri, (2019) demonstrated that exposure of rats to cadmium causes liver damage, While, Omokaro (2016), showed that lead causes liver damage in rats. This study also indicates that duration of exposure can be a factor for profound lethality in the liver, with the range of liver damage in subchronic more profound than that of acute exposure. Treatment with antioxidants: Butylated hydroxyl toluene and Vitamin E, largely modulated the severity of damages to the liver. Enzyme levels returned to near-normal in treated rats, indicating that antioxidants (BHT and Vit. E) can stabilize liver cell membranes and prevent the leakage of enzymes. However, the combined effects of BHT and Vit E had more effect in reducing the severity of the damage. This is supported by Gobe and Crane (2010), who asserted that antioxidant therapies might reduce or antagonize toxic effects of Cadmium. Vitamin E was also more potent in preventing liver damage compared to BHT in this present study.

It is a common knowledge that albumin is among plasma protein synthesized by the liver. In this study, rats treated with Cd and Pb singly and in combination had a decrease in serum and total protein and albumin concentration in comparison with the control (Table 1), this illustrates albumin and total protein production decreased in rats exposed to Pb and Cd. These results concur with El-Sokkary et al., (2010) and Omokaro, (2016), that lead and cadmium are liver damaging agents.

The significant reduction in total protein and albumin level in rats exposed to Cd and Pb exerts its effect on the synthetic function of the liver. This is in line with Kim et al. (2008), who stated that lead can cause damage to the synthetic ability of the liver. The increase in albumin and total protein level of rats treated with antioxidants, indicates that BHT and Vit. E can prevent the decrease of albumin probably through stabilizing endoplasmic reticulum and re-synthesizing protein through neutralizing ROS by scavenging compounds. Histopathological examinations (Fig. 1) confirmed the extent and near level repair by BHT and Vit. E. The liver samples from rats treated with Cd and Pb indicated damages such as necrosis, balloon formation and degeneration of biliary. However, in the groups treated with antioxidants had less degeneration and there was healing of the liver tissues.

An antioxidant is any substance, which at low concentrations compared to those of an oxidizable substrate meaningfully interrupts or thwarts the oxidation of that substrate (Kadiri, 2018). Cadmium (Cd), lead (Pb) and Cd-Pb treatment resulted in significantly (P<0.05) lower Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) and significant (P<0.05) increase in MDA levels, symbolizing oxidative stress. However, treatment with antioxidants (Groups G), resulted in significant (P<0.05) decrease in MDA levels and significant



(H&E stain, Mag. 400x).

Fig. 1: Liver Histology of Rats exposed to lead, cadmium, cadmium-lead and rats treated with Antioxidants (BHT and Vitamin E)
Key: Portal vein (PV), central vein (CV), Hepatic cell (HC), inflammation (I), necrosis (N)

($P<0.05$) increase in SOD, CAT and GPX activities. This finding is in line with Apiamu et al., (2019) who established that Cadmium has the ability to induce oxidative stress. Chiba et al., (2006), also established that lead can alter antioxidant activities by constraining functional SH groups in numerous enzymes such as SOD, CAT, and GPx. Treatment with the antioxidants (BHT and Vitamin E) both singly and in combination was able to ameliorate the Cd-Pd induced oxidative stress due to the ability of BHT and Vit E to counteract the reactive oxygen species (ROS), Also, vitamin E seems to have more ROS neutralizing capabilities when compared with BHT. The results of these experiments clearly show that exposure to heavy metals cadmium and lead can generate free radicals, it can affect macromolecules, cause liver and kidney damages and cause oxidative stress through the production of ROS, it increases MDA formation and reduces antioxidant defense system in the liver. This study has proven that, BHT and Vit. E can reduce the effect of heavy metal

Table 2: The effects of Cadmium-lead toxicity on liver SOD, CAT, GPx and MDA, in rats treated with antioxidants BHT and Vitamin E

	SOD (**Units/g wet tissues)**	CAT (u/l)	GPx (u/l)	MDA
A(Control)	26.21 ± 3.34	31.01 ± 4.04	18.34 ± 4.18	2.48 ± 0.04
Sub-acute				
B (Cd)	13.12 ± 1.04[a]	14.45 ± 5.72[a]	10.46 ± 3.07[a]	7.82 ± 0.86[a]
C (Pb)	14.41 ± 3.53[a]	15.57 ± 3.98[a]	10.20 ± 3.62[a]	7.23 ± 0.15[b]
D (Cd +Pb)	13.26 ± 2.31[a]	13.52 ± 5.10[a]	9.46 ± 3.38[a]	8.51 ± 0.15[a]
E (Cd+Pb+Vit E)	23.12 ± 2.25[bc]	25.23 ± 3.28[bc]	15.38 ± 1.70[bc]	3.00 ± 0.81[bc]
F (Cd+Pb+BHT)	21.15 ± 1.59[bc]	27.29 ± 3.24[bc]	14.18 ± 2.57[bc]	3.50 ± 0.24[bc]
G (Cd+Pb+Vit E+BHT)	24.16 ± 2.38[bc]	30.34 ± 3.11[bc]	17.15 ± 5.77[bc]	2.80 ± 0.16[bc]
Chronic				
B (Cd)	13.12 ± 2.25[a]	25.23 ± 7.28[b]	14.38 ± 1.70[b]	2.00 ± 0.81[b]
C (Pb)	13.15 ± 1.59[a]	27.29 ± 3.24[b]	11.18 ± 2.57[b]	1.50 ± 0.24[c]
D (Cd +Pb)	11.16 ± 2.38[a]	30.34 ± 8.11[a,b]	17.15 ± 5.77[a]	0.80 ± 0.16[a]
E (Cd+Pb+Vit E)	25.15 ± 2.35[bc]	25.40 ± 4.87[b]	12.33 ± 2.14[b]	1.00 ± 0.01[a]
F(Cd+Pb+BHT)	23.36 ± 2.40[bc]	23.16 ± 5.02[b]	8.59 ± 1.69[b,c]	0.60 ± 0.16[a]
G (Cd+Pb+Vit E+BHT)	26.69 ± 2.69[bc]	28.31 ± 6.36[a,b]	18.15 ± 4.22[a]	0.50 ± 0.09[a]

Values are represented in mean± SD. n=4.Startistical significance:a(P<0.05-0.001) from Normal, b(P<0.05-0.001) from Cd or Pb only, c(P<0.05-0.001) from Pb+Cd

exposure such as cadmium and lead. This protection of antioxidants against Cd and Pb may be due to the action of free radical scavengers initiated by the antioxidant defence systems, Vitamin E, a foremost lipid-soluble antioxidant and is the most active chain- terminating antioxidant inside the membrane of the cell, BHT on the other hand hunts free radicals that are known to initiate the oxidation of lipid thus halting decline processes (Ousji and Sleno 2020). A combination of these two antioxidants was able to restore the oxidative state of the animals back to near normal.

Conclusion

The present study indicates that a combination of Butylated HydroxylToluene and Vitamin E has protective effects against liver damage induced by cadmium-lead by modulating the antioxidants (SOD, CAT, GPx) and reduction of lipid peroxidation.

Acknowledgement

The Authors wish to thank Mrs Omomaife and Mrs Iwhiwhu for their technical assistance.

Conflict of Interest

The Authors declare that this work has no conflict of interest
Individual author's contributions
Conception: HEK
Design: HEK
Execution: BI, HEK
Interpretation and Analysis: HEK, BI
Writing of the Paper and Proof reading: HEK, BI

Reference

Akporido, S.O., and Kadiri, H.E. (2014). Effect of urbanization and industrialization on the waters of Benin river ethiope river system around Sapele, Nigeria, Global Advanced Research Journal of physical and Applied Sciences, 3 (3); 035-050.

Apiamu, A., Asagba, S.O., and Tonukari, N.J. (2019). Role of Anthcleista vogelii in serum antioxidant defence system in cadmium induced oxidative stress in wistar rats, Beni-Suef University Journal of Basic and Applied Science, 8(1); 1-13.

Buege, J.A., and Aust, S.D. (1978). Microsomal lipid peroxidation, Methods in Enzymology, 52; 302-305.

Chalasani, N., Younossi, Z., and Lavine, J.E. (2018). The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases, Hepatology, 67: 328-357.

Chiba, M., Shinohara, A., Matsushita, K., Watanabe, H., and Ihaba, Y. (2006). Indices of lead-exposure in blood and urine of lead-exposed workers and concentrations of major and trace elements and activities of SOD, GSH-Px and catalase in their blood, Tohoku Journal of Experimental Medicine, 178(1); 49–62.

Ellman, G.L. (1959). Tissue sulfhydryl groups. Archives of Biochemistry and Biophysics, 82:70-77.

El-Sokkary, G.H., Nafady, A.A., and Shabash, E.H. (2010). Ecotoxicology Melatonin administration ameliorates cadmium-induced oxidative stress and morphological changes in the liver of rat, Environmental Safety, 73; 456-463.

Fasihnia, S.H., Peighambardoust, S.H., Peighambardoust, S.J., Oromiehie, A., Soltanzadeh, M., and Peressini, D. (2020). Properties and Application of Multifunctional Composite Polypropylene-Based Films Incorporating a Combination of BHT, BHA and Sorbic Acid in Extending Donut Shelf-Life, Molecules, 25(21);5197-5199.

Genchi, G., Carocci, A., Lauria, G., Sinicropi M.S., and Catalano A. (2020). Nickel: Human health and environmental toxicology, International Journal of Enviromental Research and Public Health, 17; 679.

Gobe, G., and Crane, D. (2010). Mitochondria, reactive oxygen species and cadmium toxicity in the kidney, Toxicology Letters, 198(1); 49–55.

Habig, W.H. (1974). Gluthathione S-transferase: the first enzyme step in mercapturic acid formation, Gluthathione S-transferase, Journal of Biological Chemistry, 249(22); 7130- 7139.

Jonscher, K.R., Chowanadisai W. and Rucker, R.B. (2021). Pyrroloquinoline-Quinone Is More Than an Antioxidant: A Vitamin-like Accessory Factor Important in Health and Disease Prevention, Biomolecules, 11(10): 1441.

Kadiri, H.E., and Ekayoda O. (2023). Food Chain Borne Effect of Cadmium and Cyanide on Some Biochemical Indices in the Liver of Rats, Iraqi Journal of Science, 64(1):20-32

Kadiri, H.E. (2019). Combined effect of cadmium and cyanide on liver enzymes and kidney function in rats, Journal of Experimental Research, 7(1); 15-19.

Kadiri, H.E. (2018). The ameliorating effects of honey on some biochemical parameters on rats exposed to cyanide, Biokemistri, 30(1):13-20.

Kadiri, H.E., Okoro, I.O. and Ichipi-Ifukor, P. (2020). Tetrapleura Tetraptera Fruit Protects against Cyanide Induced Toxicity in Rats. Iraqi Journal of Science, 61(10):2504-2514

Kaplan, A., Dembiec, D. Cohen, G., and Marcus, J. (1972). Measurement of catalase activity in tissue extracts, Anals of Biochemistry, 34; 30-38.

Khlif R., and Hamza-Chaffai, A. (2010). Head and neck cancer due to heavy metal exposure via tobacco smoking and professional exposure: A review, Toxicology and Applied Pharmacology, 248; 71–88.

Kim, Y.J., and Korean J. (2008). Interpretation of liver function tests, Gastroenterology, Taehan Sohwagi Hakhoe chi, 51; 219–224.

Kumar, A., Kumar, A., Chaturvedi, A. K., Shabnam, A. A., Subrahmanyam, G., Mondal, R., Gupta, D. K., Malyan, S. K., S Kumar, S., A Khan, S., and Yadav, K. K. (2020). Lead Toxicity: Health Hazards, Influence on Food Chain, and Sustainable Remediation Approaches, International Journal of Environmental research and public health, 17(7); 2179.

Lorenzon Dos Santos, J., Quadros, A.S., Weschenfelder, C., Garofallo S.B., and Marcadenti, A. (2020). Oxidative Stress Biomarkers, Nut-Related Antioxidants, and Cardiovascular Disease, Nutrients, 12(3); 682-688.

Meramat, A., Rajab, N.F., and Shahar, S. (2016). DNA Damage, Copper and Lead Associates with Cognitive Function among Older Adults, Journal of Nutritional Health and Aging,21; 539–545.

Misra H.P. and Fridovich, I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a sample assay for superoxide dismutase, Journal of Biological Chemistry, 247; 3170-3175.

Okoro I.O., and Kadiri, H. E. (2019). Anti-Oxidant and Hepatoprotective Effects of Senecio biafrae on CCl4-induced Liver Damage in Rats, Iranian Journal of Toxicology, 13 (2); 31-35.

Omokaro, E.U. (2016). Effect of occupational exposure to lead on liver function parameters, International Journal of Pharmaceutical Medicine and Science, 6; 15–19.

Ousji, O., and Sleno. L. (2020). Identification of In Vitro Metabolites of Synthetic Phenolic Antioxidants BHT, BHA, and TBHQ by LC-HRMS/MS. International Journal of Molecular Science, 21(24);9525-9530.

Rahimzadeh, M.R., Kazemi, S., and Moghadamnia, A.A. (2017). Cadmium toxicity and treatment: An update. Caspian Journal of Internal Medicine, 8; 135–145.