Protection Effects of Vernonia Amygdalina Methanolic Extracts Against Hepatocellular Damage Induced by Petroleum Contaminated Diets in Male Rats

Achuba, F.I*., Ichipi-Ifukor, P. C.
Department of Biochemistry, Delta State University, PMB 1, Abraka Nigeria

Received: 4 /8/2019 Accepted: 29 /9/2019

Abstract
Recently, bitter leaf (Vernonia amygdalina) was found to prevent petroleum – induced toxicities on the kidney whereas it potentiates the toxic effect of petroleum adulterated diet on the testes of animal model. This differential action has elicited further inquest into the role of bitter leaf extract in other organs in the midst of petroleum affronts. The hepatoprotective ability of Vernonia amygdalina methanol extract (VAME) is the objective of this investigation. Administration of VAME significantly (P <0.05) reduced serum liver function indices relative to the control. In addition, the activities of liver oxidative enzymes, energy metabolizing enzymes and oxidative stress indices altered by crude oil adulterated diet were significantly (P < 0.05) reversed near control values. Similarly, VAME injection restored the histopathological indices caused by crude oil adulterated diet. It is obvious that all the liver damage indices induced by crude oil contaminated feed were prevented by the intake of VAME, indicating the hepatoprotective ability of bitter leaf

Key words: Bitter leaf, liver, lipid panel, oxidative stress

1. Introduction
It is an unhidden truth that petroleum- related resource is a major source of income in some countries of the world. However, as it generates revenue for the country, its deleterious effect to the unbundling of our environment continues to be a serious case for worry. Release of environmental petroleum-related substances occurs in several ways and manners that range from industrial oil spills in pipelines and tankers, mechanic workshops and even in some cases domestic accidents [1-4].

Today, man stands a greater risk of being consciously or unconsciously exposed to several petroleum products and its allied constituent chemicals [5,6].Several compounds related to petroleum are broken down to free radicals or highly activated metabolites that often results in several illnesses such as cancer, neurodegenerative and metabolic syndromes[7-9]. The noxiousness of environmental petroleum is highly documented as numerous reports also indicate that food borne allied exposure of petroleum to man remains a serious challenge that must be undaunted [10, 11]. Although several plant-related materials such as Monodora myristica [12]; Moringa oleifera [13]; Honey [14]; Palm oil [15,16] have been explored for controlling of food borne petroleum toxic consequences, there remains scarcity of reports on the beneficial role of bitter leaf on ameliorating food borne petroleum noxiousness in the liver.

With the understanding that the liver is the primary site of xenobiotic metabolism [17], it is justifiable to state that it will be most vulnerable to the toxic effects of food borne petroleum toxicity in man. Earlier, the beneficial role of Vernonia amygdalina had been published on kidney and haematological indices of rat [18, 19]. This report presents the ameliorative role of Vernonia
2. Materials and Methods

The bitter leaf (*Vernonia amygdalina*) was obtained from a farm at Abraka, treated and the methanolic extract was prepared and administered (at a concentration of 100 mg/pbwt and 200 mg/pbwt) as reported previously [18]. This research is a continuation to a previous study. Therefore, animal mobilisation and experimental design followed a previously published experimental design [18]. The feed composition and feeding regimen were published earlier [18].

2.1 Sample Collection and treatment

The male (*Rattus norvegicus*) rats after treatment period were sacrificed by cervical decapitation under chloroform sedation on the 31st day after an overnight fasting. The Blood and liver samples were collected and prepared for serum and tissue homogenate analysis as previously reported [18].

2.2 Biochemical Analysis

Determination of serum AST, ALT, ALP, ACP, Glucose, Total Protein, Albumin, Globin and Lipid Profile (Cholesterol, TAG, HDL and LDL) were done using commercial Randox diagnostic kits. While Glucose-6-phosphate dehydrogenase activity was determined following the method of Henry [21]. All other reagents used for biochemical assay were of analytical grades and employed the use of the experimental procedures such as Gutteridge and Wilkins [20] method for lipid peroxidation (MDA), Omarov et al. [22], D’Errico et al. [23] and McEwen [24] for aldehyde oxidase (AO), sulphite oxidase; xanthine oxidase (XO) and monoamine oxidase (MO) activities respectively. The level of reduced glutathione in the liver was determined using the procedures of Ellman [25] while vitamin C estimation employed the method reported previously [26]. The following methods were adopted during the determination of enzymatic antioxidants activities, which were Misra and Fredorich [27], Cohen et al. [28] Habig et al. [29], Khan et al. [30] for superoxide dismutase (SOD), Catalase (CAT), glutathione-s-transferases (GSTs) and glutathione peroxidase (GPx) activities, respectively.

2.3 Histological Analysis

The examination of the liver tissues followed the method of Al-Attar et al. [31] which has been described in detail by Achuba [18].

2.4 Statistical Analysis

The Statistical Package for Social Sciences (SPSS 17) was employed to perform analysis of variance (ANOVA) and Post hoc examination (multiple groups comparisons) performed with Bonferroni at p<0.05 significance point.

Results and Discussion

The continual search for antidote for petroleum toxicity remains an onus task for scientists as people living in oil producing areas of the world contend with several illnesses arising from petroleum pollution and cross contaminations [32, 33]. Evidences from this present study indicates that the consumption of petroleum contaminated diets elevated the various liver function enzymes in the serum (AST, ALT, ACP, ALP) while reducing serum glucose, total protein, albumin and globulin levels compared to rats that did not consume petroleum tainted diets (Table-1).
Table 1 - Effect of VAME on serum biochemical liver function parameters of rats fed petroleum contaminated diet

|       | A                | B                | C                | D                | E                | F                |
|-------|------------------|------------------|------------------|------------------|------------------|------------------|
| AST(UL⁻¹) | 58.98±0.59ᵃ      | 53.47±1.03ᵇ      | 56.05±0.57ᵇ      | 69.94±1.61ᶜ      | 54.17±10.35ᶜ     | 57.09±7.76ᵈ      |
| ALT(UL⁻¹) | 41.62±0.94ᵃ      | 40.97±1.48ᵇ      | 44.99±1.21ᵇ      | 59.33±3.92ᶜ      | 43.70±2.89ᵈ      | 48.09±4.65ᵈ      |
| ACP(UL⁻¹) | 4.48±0.36ᵃ       | 5.87±0.56ᵇ       | 5.07±0.88ᵇ       | 11.88±0.61ᵈ      | 7.99±1.62ᶜ       | 7.82±1.27ᶠ       |
| ALP(UL⁻¹) | 112.63±0.83ʳ     | 137.50±1.29ˢ     | 126.77±2.61ᵇ     | 130.94±1.00ᶜ     | 121.50±5.20ᵇ     | 142.01±5.51ᶜ     |
| Glucose(gdl⁻¹) | 6.30±0.37ᵃ     | 133.00±0.82ᵃ     | 132.25±1.71ᵇ     | 4.70±0.18ᵃ⁴     | 123.67±0.57ᵇ     | 119.50±1.29ᵇ     |
| TotalProtein(gdl⁻¹) | 6.45±0.13ᵃ      | 6.25±0.13ᵃ⁴     | 5.77±0.15ᵇ       | 5.65±0.33ᵇ      |                  |                  |
| Albumin(gdl⁻¹) | 3.55±0.13ᵃ      | 3.70±0.14ᵃ      | 3.18±0.13ᵇ       | 2.50±0.22ᵃ⁵     | 3.10±0.10ᵇ      | 2.66±0.13ᵃ      |
| Globin(gdl⁻¹) | 2.70±0.08ᵃ       | 2.55±0.34ᵃ      | 3.00±0.18ᵃ⁴     | 1.40±0.37ᵇ      | 2.77±0.15ᵃ⁵     | 2.93±0.22ᵃ⁵     |

Group A=Feed; Group B=Feed+100 mg kg⁻¹ body weight of VAME; Group C=Feed+200 mg kg⁻¹ body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg⁻¹ body weight of VAME; Group F= Tainted feed +200 mg kg⁻¹ body weight of VAME.

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference.

There is no doubt that petroleum contamination has been severely implicated in the up regulation of liver function enzymes such as the transferases and phosphatases [14, 34]. The observed up-regulation of the liver enzymes (transferases and phosphatases) and the concomitant reduction in serum protein levels may be indicative of critical threats to the liver integrity and is similar to the once reported by Okpoghono et al.[35], Achuba and Ogwumu [16] who reported on the consumption of petroleum contaminated cat fish meal and petroleum tainted diet an upsurge in these enzymes and reduction in the serum proteins. Treatment with both doses (100mKg⁻¹ and 200mgKg⁻¹) body weight of Bitter leaf methanolic extract was observed to mitigate the liver enzymes while modulating the total protein, albumin and serum glucose levels. This observation is consequential with reports made in previous studies as to the ability of plant materials to mitigate the upsurge in AST, ALT, ACP, ALP as reported by Achuba et al. [13], Okpoghono et al.,[35] and Achuba and Ogwumu [16].

Serum lipid profiling has been identified as a very significant marker for possible cardiovascular disorders [13, 36]. Likewise serum lipid profile has also been used as a significant marker for the integrity of liver cells [35]. The observed drop in total cholesterol, triglyceride, HDL-cholesterol and concomitant rise in LDL-cholesterol in rats consuming only petroleum contaminated diets and rats treated with both doses of VAME after consumption of petroleum tainted diets compared to normal control (Table-2) is in line with the report of Achuba and Otuva [37] who reported the ability of antioxidant vitamins to modulate positively the lipid panel of rats fed with petroleum tainted diet.
Table 2-Effect of VAME on serum lipid profile of rats fed petroleum contaminated diets

|       | A               | B               | C               | D               | E               | F               |
|-------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Cholesterol (mg/dl) | 104.25±0.96<sup>a</sup> | 101.50±1.29<sup>b</sup> | 101.50±1.29<sup>b</sup> | 115.25±0.96<sup>c</sup> | 111.00±1.00<sup>c</sup> | 105.00±0.82<sup>d</sup> |
| TAG (mg/dl)        | 130.50±1.91<sup>a</sup> | 123.00±2.58<sup>b</sup> | 108.25±2.06<sup>c</sup> | 126.25±2.36<sup>b</sup> | 127.00±1.00<sup>c</sup> | 124.25±1.25<sup>d</sup> |
| HDL (mg/dl)        | 18.25±0.96<sup>a</sup> | 13.00±0.82<sup>bc</sup> | 12.75±1.25<sup>b</sup> | 14.00±0.82<sup>ac</sup> | 18.00±1.00<sup>ac</sup> | 15.50±1.29<sup>c</sup> |
| LDL (mg/dl)        | 40.03±24.96<sup>c</sup> | 33.33±0.73<sup>b</sup> | 35.05±1.10<sup>b</sup> | 43.50±0.51<sup>b</sup> | 36.17±2.86<sup>b</sup> | 37.27±1.96<sup>b</sup> |

Group A=Feed; Group B=Feed+100 mg kg<sup>−1</sup> body weight of VAME; Group C=Feed+200 mg kg<sup>−1</sup> body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg<sup>−1</sup> body weight of VAME; Group F= Tainted feed +200 mg kg<sup>−1</sup> body weight of VAME.

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference.

Several plant materials have been reported to contribute to eventual reversal of such trends near control values [13, 38, 39]. The trend in the observations made in this study further gives credence to an earlier implication of extracts of bitter leaf on lipid profiles [40].

Another significant observation in this study was the fluctuations in key enzymes of energy metabolism (Figure-1). The observed drop in glucose 6 phosphate dehydrogenase and increased lactate dehydrogenase activities in rats fed only petroleum contaminated diets and those treated with both doses of VAME after consumption of petroleum polluted diets relative to the control group may be indicative of a starve in energy demand owing to the toxicity impact of petroleum diet consumption and the eventual counter-effective functions of the VAME.
Group A=Feed; Group B=Feed+100 mg kg$^{-1}$ body weight of VAME; Group C=Feed+200 mg kg$^{-1}$ body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg$^{-1}$ body weight of VAME; Group F= Tainted feed +200 mg kg$^{-1}$ body weight of VAME.

**Figure 1**-Effect of VAME on key enzymes of energy metabolism in the liver of rat fed crude oil contaminated diet

It is important to note that the G6DPH is a significant enzyme catalyzing the oxidation of glucose 6 phosphate in the pentose phosphate partway while contributing to the supply of reducing energy potentials to cells for the continuation of the energy cycle and to tissues involved in the biosynthesis of fatty acids [41-43]. This drop in the G6DPH activity could be the justification of the increased activities of LDH which could be said to have been triggered for the promotion of anaerobic respiration for replenishing the increased depletion of NADPH within the cells which has been cut-short by possible inhibition of the G6DPH. A further drop in the SDH activities also gives credence to our earlier claim and suggestion of possible drop in energy supply owing to shortfall of certain metabolites. Succinate dehydrogenase has been reported to be a very significant enzyme in the citric acid cycle (TCA) and the electron transport chain (ETC). In the TCA cycle, it catalyses the oxidation of succinate to fumarate which eventually supplies the FADH$_2$ needed for the reduction of ubiquinone to ubiquinol [44-46]. By implication therefore, the alternative promotion of LDH activities within the metabolic cycle could be said to have not been effective enough for the supply of the needed substrates that promotes the TCA cycle hence drop in the SDH activities.

Increase in lipid peroxidation (MDA) and activities of the oxidative enzymes (AO, SO, MO and XO) has been noted as very significant indicators of metabolic stress (Table-3).
Table 3: Effect of VAME on lipid peroxidation and oxidative enzyme activities in the liver

| Groups | MDA $\mu$mol ml$^{-1}$ | AO Units g$^{-1}$ tissue | SO Units g$^{-1}$ tissue | MO Units g$^{-1}$ tissue | XO Units g$^{-1}$ tissue |
|--------|------------------------|------------------------|------------------------|------------------------|------------------------|
| A      | 40.19±0.49$^a$         | 76.00±3.65$^a$         | 666.75±28.55$^a$       | 157.75±12.55$^a$       | 62.25±1.71$^d$         |
| B      | 40.58±0.67$^a$         | 85.75±5.38$^b$         | 705.25±32.55$^{ab}$    | 166.00±11.17$^{ab}$    | 66.70±1.71$^{ab}$      |
| C      | 44.96±0.96$^b$         | 89.50±5.44$^b$         | 747.25±19.24$^b$       | 182.75±2.98$^b$        | 70.50±2.08$^{bc}$      |
| D      | 43.65±1.16$^{bc}$      | 93.00±2.94$^b$         | 772.25±13.67$^{bc}$    | 189.75±2.74$^{bc}$     | 73.50±2.38$^c$         |
| E      | 42.38±1.78$^{ab}$      | 106.00±7.70$^c$        | 790.25±12.71$^{bc}$    | 193.75±4.35$^{bc}$     | 76.00±2.16$^d$         |
| F      | 41.37±1.51$^{ac}$      | 119.00±3.56$^d$        | 804.75±5.37$^c$        | 203.00±5.10$^c$        | 80.00±2.16$^d$         |

Group A=Feed; Group B=Feed+100 mg kg$^{-1}$ body weight of VAME; Group C=Feed+200 mg kg$^{-1}$ body weight of VAME; Group D=Tainted feed; Group E=Tainted feed +100 mg kg$^{-1}$ body weight of VAME; Group F=Tainted feed +200 mg kg$^{-1}$ body weight of VAME

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference

The increased MDA levels in rats only fed petroleum tainted diets is in response to the generation of reactive oxygen species while the rise in the oxidative enzymes occurred as a result of a second messenger response for the clearance of the oxidative radicals generated within the tissues so as to help reduce the rising MDA and is in agreement with the studies of Okpoghono et al. [35]; Achuba et al. [13]. Although treatment with both doses of VAME further led to a rise in MDA levels and increase in oxidative enzyme activities this must have occurred as a result of the abilities of VAME to promote or induce the oxidative enzyme activities to rise up to the increasing challenge of petroleum induced oxidative stress. On the other hand rising lipid peroxidation and increase in ROS has been reported to be a significant inducer of several antioxidant enzymes to aid the clearance of the oxidative radicals. Such enzymes as SOD, catalase, GPx and GSTs are known buffers for oxidative stress defense systems. Other non-enzymatic antioxidants such as vitamin C and GSH are also said to contribute to this oxidative buffering system but may be depleted when overpowered by a high degree of ROS generation. As in other studies, there were observed depletion in non-enzymatic antioxidants Vitamin C and GSH as well as catalase, and GSTs activities while GPx increased in rats consuming only petroleum polluted diets compared to control (Table-4).
Table 4 - Effect of VAME on some enzymatic and non-enzymatic antioxidant profiles in liver

|       | Vit.C mgg⁻¹Fwt | GSH µmolm⁻¹protein | CAT µmolm⁻¹protein | GPx µmolm⁻¹protein | GSTs µmolm⁻¹protein |
|-------|-----------------|--------------------|-------------------|-------------------|--------------------|
| A     | 4.13±0.44a      | 0.37±0.84a         | 168.06±1.99a      | 0.39±0.03a        | 390.75±29.48a      |
| B     | 4.83±0.17a      | 0.41±0.72a         | 170.20±1.76a      | 0.41±0.03ac       | 418.25±39.28ab     |
| C     | 4.93±0.30a      | 1.57±2.29a         | 213.04±1.46b      | 0.92±0.04b        | 455.25±21.75b      |
| D     | 3.55±0.34a      | 0.31±0.06c         | 162.99±0.70c      | 0.78±0.07b        | 343.00±8.04c       |
| E     | 3.45±0.31a      | 0.29±0.05c         | 113.57±1.10b      | 0.80±0.07b        | 359.00±18.31c      |
| F     | 2.18±0.66c      | 0.23±0.05c         | 112.32±0.51d      | 0.55±0.09c        | 316.00±10.86c      |

Group A= Feed; Group B= Feed+100 mg kg⁻¹ body weight of VAME; Group C= Feed+200 mg kg⁻¹ body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg⁻¹ body weight of VAME; Group F= Tainted feed +200 mg kg⁻¹ body weight of VAME.

All data stated as Mean± SEM. Values with different superscript in each column designates a significant difference.

Treatment with both doses of VAME alone was also observed to have boosted the levels of these antioxidants, post-treatment of VAME after petroleum diet consumption was observed to have further declined compared to control and the rats not treated after petroleum diet consumption (Figure 2).

![Figure 2](image_url)

Group A= Feed; Group B= Feed+100 mg kg⁻¹ body weight of VAME; Group C= Feed+200 mg kg⁻¹ body weight of VAME; Group D= Tainted feed; Group E= Tainted feed +100 mg kg⁻¹ body weight of VAME; Group F= Tainted feed +200 mg kg⁻¹ body weight of VAME.

Figure 2: Effect of VAME on Liver CuZnSOD, MnSOD and total SOD activities. Different alphabet different superscripts on bars of the colour designate a significant difference.
The possible justification for this trend is that although VAME contributed to antioxidant defense, the veracity of ROS generation due to petroleum metabolism within the liver tissue may have overpowered the continual upsurge in the antioxidant defense hence the eventual depletion. In giving further explanation to this phenomenon various authors submitted that metabolic stress inducers are also significant inducers of several antioxidant and oxidative enzymes which act competitively until either of them (stressor or antioxidant) is overpowered to achieve metabolic stability or instability[47-49].

**Figure 3A-F:** Photomicrographs of rat liver fed petroleum contaminated diets and treated with bitter leaf extract. Haematoxylin and Eosin x 100 magnification.
The liver histology revealed that rats administered only petroleum contaminated diet indicates a very visible distortion of the liver architecture which gives further credence to the observed depletion in antioxidant defence systems (Figure-3A-F). Treatment with both doses of BLME conferred protection on the liver integrity; this is in agreement with similar observations made in the kidney by Achuba [18] which also reported the prevention of petroleum induced kidney necrosis by BLME. This further gives credence to earlier report by Kambizi and Afolanya [50] on the efficacy of bitter leaf.

Conclusions
The present study further established the role of petroleum contaminated diet consumption in the induction of several metabolic anomalies; it also showed the ability of VAME to confer certain levels of protection to the liver on the outcomes of these anomalies.

References
1. Burns, K.A., Garry, S.D, Jorissen, D., MacPherson, J., Stoelting, M., Tierney, J, Yelle-Simmons, L.1994. The Galatea oil spill. II. Unexpected persistence of oil trapped in mangrove sediments. Estuary Coast Shelf Science, 38: 349–364.
2. Reddy, C.M., Eglinton, T.I., Hounshell, A., White, H.K., Xu, L., Gaines, R.B.and Frysinger, G.S.2002. The West Falmouth oil spill after thirty years: the persistence of petroleum hydrocarbons in marsh sediments. Environmental Science and Technology, 36: 4754–4760
3. Short, J.W., Lindeberg, M.R., Harris, P.M, Maselko JM, Pella, JI, Rice, S.D.2002 Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. Environmental Science and Technology, 38: 19–25
4. Mahmoud, K., Shalahmetova, T. and Deraz, S.2011. Effect of Crude oil Intoxication on Antioxidant and Marker Enzymes of Tissue Damage in Liver of Rat International Journal of Biotechnology and Bioengineering, 5(11) :650-652
5. Patrick-Iwuanyanwu, K. C., Onyymaenu, C.C., Wegwu, M.O. and Ayalogu, E.O.2011. Hepatotoxic and nephrotoxic effects of kerosene and petrol-contaminated diets in Wistar albino rats. Journal of Environmental Toxicology, 5(1): 49 – 57.
6. Almeda, R., Wambaugh, Z., Chai, C., Wang, Z., Liu, Z.and Buskey, E.J.2013. Effects of crude oil exposure on bioaccumulation of polycyclic aromatic hydrocarbons and survival of adult and larval stages of gelatinous zooplankton. PLoS One 8:e74476https://doi.org/10.1371/journal.pone.0074476
7. Odo, C.E., Nwodo, O.F.C., Joshua, P.E., Ubani, C.S., Etim, O.E. and Ugwu, O.P.C.2012. Effects of bonny light crude oil on anti-oxidative enzymes and total proteins in Wistar rats. African Journal of Biotechnology, 11(98):16455-16460.
8. Onwurah, I.N.E. and Eze, O.2007. Superoxide dismutase activity in Azotobacter vinelandii in the disposition of environmental toxicants exemplified by fenton reagent and crude oil. Journal of Toxic Substances 79:111-123.
9. Ogara, A.L., Joshua, P.E., Omeje, K.O.and Onwurah, I.N.E.2016. Effects of Ingested Crude Oil Contaminated Diets on Antioxidant Enzyme and Lipid Profile in Wistar Albino Rat. Journal of Applied Science and Environmental Management, 20 (4) 927-932, http://dx.doi.org/10.4314/jasem.v20i4.4
10. Uboh, F.E., Ufot, S.U.and Eyon, E.U.2013. Comparative effect of withdrawal from exposure on gasoline and diesel induced nephrotoxicity in male albino wistar rats. Journal of Clinical Toxicology, 3:170. https://doi.org/10.4172/2161-0495.1000170.
11. Azeez, O.M., Akhigbe, R.E.and Anigbogu, C.N.2013. Oxidative status in rat kidney exposed to petroleum hydrocarbons. Journal of Natura Science, Biology and Medicine , 4:149–154. https://doi.org/10.4103/0976-9668.107280
12. Okpogono, J., Achuba, F.I and George, B.O. 2018a. Influence of Monodora myristica extracts on Lipid Profile of Rats Fed Crude Petroleum Oil Contaminated Cattish Diet. Sokoto Journal of Medical Laboratory, Science 3(1): 19-27.
13. Achuba, F.I., Uboegu, L.A. and Ekute, B.O.2018. Moringa Oleifera Treatment Prevents Crude Oil Tainted Diet Imposed Toxicity in Rats Sokoto Journal of Medical Laboratory Science, 3(3): 99 -105.
14. Achuba, F.I. and Nwokogha, C.C. 2015. Effects of honey supplementation on hydrocarbon-induced kidney and liver damage in Wistar albino rats. *Biokemistri*, 27(1): 50–55.
15. Achuba, F.I. 2018a. Modulation of crude oil induced alteration of oxidative stress indices in rat by red palm oil. *Journal of Applied Science and Environmental Management*, 22(6): 929-932. https://dx.doi.org/10.4314/jasem.v22i6.15
16. Achuba, F.I. and Ogwumu, M.D. 2014. Possible protective role of palm oil and beef liver on the kidney and liver of wistar albino rats fed diesel-contaminated diet. *Biokemistri*, 26(4): 124–129.
17. McKee, T. and McKee, J.R. 1999. *Biochemistry: An introduction*. McGraw-Hill Education.
18. Achuba, F.I. 2018b. Role of bitter leaf (*Vernonia amygdalina*) extracts in prevention of renal toxicity induced by crude petroleum contaminated diets in rats. *International Journal of Veterinary Science and Medicine*, 6(2): 172-177
19. Achuba, F.I. 2018c. Evaluation of the protective effects of bitter leaf (*Vernonia amygdalina*) on haematological indices of rats fed with crude oil treated diet. *Journal of Applied Sciences and Environmental Management*, 22(4): 489-492. https://dx.doi.org/10.4314/jasem.v22i4.8
20. Gutteridge, J.M.C. and Wilkins, C. 1982. Copper dependent hydroxyl radical damage to ascorbic in rats. *FEBS Letters*, 137: 327–40.
21. Henry, J.B. 1979. *Clinical Diagnosis and Management by Laboratory Methods*. W.B. Saunders and Company, Philadelphia, PA p. 365
22. Omarov, R.T., Sagi, M. and Lips, S.H. 1998. Regulation of aldehyde oxidase and nitrate reductase in roots of barley (*Hordeum vulgare* L.) by nitrogen source and salinity. *Journal of Experimental Biology*, 49: 897-902.
23. D’Errico, G., Di Salle, A., La Cara, F., Rossi, M., and Cannio, R. 2006. Identification and characterization of a novel bacterial sulfite oxidase with no heme binding domain from Deinococcus radiodurans. *Journal of bacteriology*, 188(2): 694–701.
24. McEwen, C.M. 1971. Monoamine oxidase (human serum or plasma). In: Colowick, S.P. and Kaplan, N.O. (Editors). *Methods in Enzymology*. New York: Academic Press; 1971. pp. 692–693.
25. Ellman, G.C. 1959. Tissue sulphydryl groups. *Archieve of Biochemistry and Biophysics*, 82: 70–7
26. Achuba, F.I. 2008. African land snail Achatina marginatus, as bioindicator of environmental pollution. *North Western Journal of Zoology*, 24(1):1–5.
27. Misra, H.P. and Fridovich, I. 1972. The role of superoxide ion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, 247: 3170-3175.
28. Cohen, H.J., Betcher–Lange, Kessler, D.L. and Rajagopalan, K.V. 1972. Hepatic sulphite oxidase congruency in mitochondria of prosthetic groups and activity. *Journal of Biological Chemistry*, 247(2): 7759-7766.
29. Habig, W.H., Pabst, M.J, and Jakoby, W.B. 1974. Glutathione -s-transferases: first enzymic step in mercapturic acid formation. *Journal of Biological Chemistry*, 249: 7130–7139.
30. Khan, M.R., Rizvi, W., Khan, R.A., Sheen, S. 2009. Carbon tetrachloride induced nephrotoxicity in rats: protective role of *Digeria muricata*. *Journal of Ethnopharmacology*, 122:91–99.
31. Al-Atta,r A.M , Alrobai, A.A. and Almalki, D.A. 2017. Protective effect of olive and juniper leaves extracts on nephrotoxicity induced by thioacetamide in male mice. *Saudi Journal of Biological Science*, 24(1): 15-22
32. Achuba, F.I. 2018d. powdered oil palm (*Elaeis guineensis* Jacq) leaf as remedy for hydrocarbon induced liver damage in rats. *Nigerian Journal of Pharmaceutical and Applied Science Research*, 7(3): 89-95
33. Ordinioha, B. and Brisibe, S. 2013. The human health implications of crude oil spills in the Niger delta, Nigeria: An interpretation of published studies. *Nigerian Medical Journal*, 54(1): 10-16.
34. Momoh, J. and Oshin, T.T. 2015. Severe hepatotoxicity and nephrotoxicity of gasoline (petrol) on some biochemical parameters in Wistar male albino rats. *American Journal of Biochemistry*, 5: 6-14.
35. Okpogholo, J., Achuba, F.I. and George, B.O. 2018b. Protective effect of *Monodora myristica* extracts on crude petroleum oil contaminated catfish (*Clarias gariepinus*) diet in rats. *International Journal of Veterinary Science and Medicine*, 6(1): 117-122.
36. Ujowundu, C.O., Nwokedinobi, N., Kalu, F.N., Nwaoguikpe, R.N., Okechukwu, R.I. 2011. Chemoprotective potentials of *Ocimum gratissimum* in diesel petroleum-induced hepatotoxicity in albino wistar rats. *Journal of Applied Pharmaceutical Sciences*, 1(10): 56-61.
37. Achuba, F.I. and Otuya, E.O. 2006. Protective Influence of Vitamins against Petroleum-Induced Free Radical Toxicity in Rabbit. *The Environmentalist, 26*: 295-300.

38. Achuba, F.I. 2018e. Protective Influence of Elaesis guineensis leaf in diet on petroleum-mediated kidney damage in rat. *Nigerian Journal of Pharmaceutical and Applied Science Research, 7*(2): 33-38.

39. Okpoghono, J; George, B.O. and Achuba, F.I. 2018c. Assessment of antioxidant indices after incorporating crude oil contaminated catfish and African nutmeg (*Monodora myristica*) extracts into rat diet. *Journal of Applied Science and Environment Management, 22*(2): 197 – 202.

40. Adaramoye, O.A., Akintayo, O., Achem, J. and Fafunso, M.A. 2008. Lipid-lowering effects of methanolic extract of *Vernonia amygdalina* leaves in rats fed on high cholesterol diet. *Vascular Health Risk Management, 4*(1): 235–41.

41. Aster, J., Kumar, V., Robbins, S.L., Abbas, A.K., Fausto, N. and Cotran, R.S. 2010. Robbins and Cotran pathologic Basis of Disease. Saunders/Elsevier. Kindle Locations pp.3340–3341.

42. Cappellini, M.D. and Fiorelli, G. 2008. Glucose-6-phosphate dehydrogenase deficiency. *Lancet, 371*(9606): 64–74.

43. Kotaka, M., Gover, S., Vandeputte-Rutten, L., Au, S.W., Lam, V.M. and Adams, M.J. 2005. Structural studies of glucose-6-phosphate and NADP+ binding to human glucose-6-phosphate dehydrogenase. *Acta Crystallographica D, 61*(Pt 5): 495–504.

44. Oyedotun, K. S. and Lemire, B.D. 2004. The quaternary structure of the Saccharomyces cerevisiae succinate dehydrogenase. Homology modeling, cofactor docking, and molecular dynamics simulation studies. *The Journal of Biological Chemistry, 279*(10): 9424–31.

45. Sun, F., Huo, X., Zhai, Y., Wang, A., Xu, J., Su, D., Bartlam, M. and Rao, Z. 2005. Crystal structure of mitochondrial respiratory membrane protein complex II. *Cell, 121*(7): 1043-1057.

46. Tomitsuka, E., Hirawake, H., Goto, Y., Taniwaki, M., Harada, S. and Kita, K. 2003. Direct evidence for two distinct forms of the flavoprotein subunit of human mitochondrial complex II (succinate-ubiquinone reductase). *Journal of Biochemistry, 134*(2): 191–195.

47. Kweki, G.R., Ichipi-Ifukor, P.C. and Asagba, S.O. 2018. High Caffeine-Containing Energy Drink-Induced Metabolic Stress in Rats. *Sokoto Journal of Medical Laboratory Sciences, 3*(3): 86-93.

48. Ichipi-Ifukor, P.C, Evwhe, O.L. and Eferobohwo, A. 2013. A comparative study of the effect of cadmium and arsenic on the activities of brain sulphite oxidase and aldehyde oxidase in the african cat fish (Clarias gariepienus) *Journal of Research in Bioscience, 9*(1): 68-71.

49. Asagba, S.O. 2010. Alteration in the activity of oxidative Enzymes in the tissues of male wistar Albino rats exposed to cadmium. *International Journal of Occupational Medicine and Environmental Health, 85*:1033–1041.

50. Kambizi, L. and Afolayan, A.J. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. *Journal of Ethnopharmacology, 77*(1): 5-9.