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EFFECT OF *VERONIAAMYGDALINA* ON THE ACTIVITIES OF HEPATIC ENZYMES IN WISTAR ALBINO RAT FED WITH CRUDE OIL CONTAMINATED DIET

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ABSTRACT

The present study was carried out to determine the effect of *Veroniaamygdalina* on the liver of rats fed with crude oil contaminated diet. Preliminary toxicity study was carried out to determine the concentration of bitter leaf and crude oil that could cause toxicity and was carried out using 24. healthy male albino rats that were grouped into 6 of 4 each: Group A animals were the control group fed with just 100g of feed and Group B animals were fed with 100g of feed and 5g of biter leaf; while group C were fed 100g of feed and 10g of bitter leaf, group D were fed 100g of feed 5g of bitter leaf and4ml of crude oil, group B were fed with 100 of feed 10g of bitter leaf and 4ml of crude oil and group F were fed with 100g of feed and 4ml of crude oil. All the plasma liver biomarker enzymesAST, ALT, ALP and ACP were significantly increased ($P<0.05$) in all the grouped rats i.e. group B to group F compared to the group A rats which were the 'control but elevated levels of the liver biomarker enzymes were noticed in the group F rats fed with just crude oil contaminated diet. This is an indication of impaired liver function. Aggressiveness, loss of body hairs and weight was also noticed which are clear indications of toxicity.

Key words:*Veroniaamygdalina*, Contaminated Diet, Hepatic, Enzymes, Wistar, Albino Rat, Crude Oil

Introduction

*Veroniaamygdalina*is a shrub that has been commonly known as bitter leaf due the bitter taste of the leaves. It is widely used as a daily green vegetable or herb to treat malaria and diabetes. The potential of *V. amygdalina* was first noted when scientists observed chimpanzees use the pith of this shrub for self-deparasitization. Since that discovery, subsequent researches had unveiled.

The usage of *V. amygdalina* as medicinal herb started when zoo pharmacologists found that sick chimpanzees with empty stomach sucked pith and juice from the unsavory Vernonia plant stalk (which was, not their common diet) for self-deparasitization, enhanced body fitness, increased strength or appetite and reduced constipation or diarrhoea especially during rainy season(Clayton and Wolfe, 2007; Huffman and Seifu, 2005;Huffman *et al*;, 2000; Jisaka*et al*;, 1993ab; Koshimizu*et al*., 2004).The bitter taste of *V. amygdadalina* was suspected as a guide for them to choose for the appropriate plant, plant part and amount of intake (Koshimizu*et al*, 2007). Other than animals, some of the citizens in Africa especially parents who were less educated with low or middle income also liked to use this plant, due to cultural and economic reasons (Amira and Okubadejo, 2007). More and more bioactivities possessed by different extracts of this plant such as anti – diabetic, anti-bacterial, anti-malaria, anti-fungal, anti-oxidant, liver protection and cytotoxic effects which are beneficial to health. Compounds including steroid glycosides, sesquiterpene lactones and flavonoids which contributed to its bitter taste and bio-activities have also been isolated from this plant. Toxicology studies documented on this plant shows that *V. amygadalina* low or no toxicity hereby supporting the safe use of this plant for the benefits of health.

Crude oil has been described as a complex mixture of over 6,000 potentially different hydrocarbons and metals (Edwards, 2007). The behavior of crude oil is determined by their chemistry. The main constituents of crude oil can be grouped into several broad classes of compounds: saturates (including waxes), aromatics, resins and asphaltenes. Saturates are alkanes with structures of CnH2n+2 (aliphatic) or CnH2n in the case of cyclic saturates (alicyclics). Crude oil exploration/ exploitation is known worldwide for its attendant environmental and social concerns at the local level such as loss of indigenous farm lands, destruction of rainforests, contamination of water sources and air pollution, etc (Adeola, 2015; Iruonage, 2008).

The devastating consequences of oil spill with its eventual hazards on both aerial and terrestrial environment manifest as an irreversible chain effect on both the biodiversity and human safety (Narayanan, 2007). As this occurs, the oil threatens surface water and a wide range of subsurface marine organisms which are linked in a complex food chain (Katwijik

Van *et al*., 1999). Oil spillage has caused destruction of food resources (Percival and Evans, 1997) Animal species that are not directly in contact with the oil spillage can also be harmed via the food web and predators that consumed contaminated marine preys. A variety of pollutants including crude oil and its products are known to induce stress conditions which impair the health of animals. (Ekweozor, 2002) reported that frequent spillages of crude oil and its products in creeks and rivers of the Niger Delta have resulted in a marked reduction in the number of both fresh water and marine creatures.

Most of the lands in oil producing areas of Nigeria are used for cultivation because the main occupation of people living in the region are farming and fishing (Egborge, 2013). In land used for agricultural purposes, petroleum or diesel contaminated wastes and accidental spills of crude oil at some drilling sites pose exposure risks for occupational public, livestock and wildlife (Khan *et al*., 2001). The petroleum hydrocarbons can eventually get into man and animals through ingestion of contaminated food or bio concentration through the food chain (Jessup and Leighton, 2009).

The ingestion of petroleum has been reported to induce oxidative stress Val and Almeda-Val, 2008) through the generation of free radicals (Achuba and Osakwe, 2003). It has been established that free radical generation with subsequent oxidative modification leads to lipid peroxidation Halliwell, 2006) that damages critical cellular macromolecules such as DNA, lipids and proteins (Breimer, 2000; Romero *et al*., 2002; Souza *et al*., 2012) and results in inactivation of antioxidant enzymes (Pigeolet*et al.,* 2013).

Materials and Methods

Study Area

The crude oil was obtained from Platform Petroleum Limited, Ebedei Field, Delta State, Nigeria. While the bitter leaf was gotten from a farm in Abraka in Ethiope West Local Government Area of Delta state.

Sample collection

After 30 days of feeding all the animals were sacrificed and, blood samples were taking from the animals using a 2m1 syringe, the livers were removed, weighed and stored in Formaldehyde solution.

Animals

The animals. for this experiment, Wistar albino rats, aged between 3 to 4 months were acquired from an animal house in Abraka, Delta state.

Methods

Preparation of bitter leaf

The bitter leaf was dried, ground and stored in containers

Experimental design

Different measurement of crude oil contaminated diets was prepared with bitter leaf incorporated into some of them by weighing out definite amounts of animal feed, bitter leaf and crude oil and mixed thoroughly.

Wistar albino rats weighing between 120 and 200g, were maintained on a commercial feed (growers mash) for about seven days in the animal house before the commencement of the experiment for acclimatization.

The animals were divided into six (6) different cages of four (4) rats each with the cage 1 being the control and 'hey got acclimatized to laboratory conditions, feeding regimes and handling procedures for one week, i.e. 7 days, before the commencement of the experiment. They were fed on commercial growers' mash and water.

Cage A. which was the control group contained no crude oil or bitter leaf in their diet (i.e. 0% group) and they were fed with 100g of feed,

Cage B. were fed with 100g of feed and 5g of bitter leaf,

Cage C. were fed 10g of feed and l0g of bitter leaf,

Cage D: were fed l00g of feed, 5g of bitter leaf and 4ml of crude oil,

CageE. were fed l00g of feed, l0g of bitter leaf and 4m1 of crude oil,

Cage F. were fed l00g of feed and 4m1 of crude oil.

Animal treatment

After every two (2) days the cages were washed, dried and the saw dust used in the cage to keep the animals warm was changed to avoid maggots and other microorganism to thrive, the animals were given adequate feed daily throughout the period of the experiment.

Experimental Determination of Parameters

Serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities as well as serum creatinine and urea were determined using RANDOX KITS purchased from Randox Laboratory Limited, 55 diamond road, Crumlin, United Kingdom.

Determination of Aspartate Amino Transferase Activity (Ast). Method

Aspartate aminotransferase activity was determined using the method specified in the randox kits.

Principle:

A- keioglutarate + L- aspartate. - L-glutamate + oxaloacetate. AST is measured by monitoring the concentration of oxaloacettehydazone formed with 2, 4-dinitrophenyihydrazine.

Sample material:

(i) Serum

Reagent Preparation

Sodium hydroxide solution (O.4mol Jl)

16.0g of sodium hydroxide was dissolved in distilled water and made up to 1 litre

Table 1 Reagent composition

Reagent 1	Contents	Initial concen6tration of sodium
	Buffer; phosphate buffer	(100mmmo1/1, pH 7.4)
	L -aspartate	(100mmo1/L)
	A – Ketoglutarate	(2mmo1/L)
Reagent 2	2,4 – dinitropheny1-hydrazine	2mmo1/L)

Procedure:

0.1ml of the sample was pipette into test-tubes, 0.5 ml of reagent 1 was added and

mixed. The mixture was incubated for 30 minutes at 37°C. Reagent 2was added and solution was allowed to stand for 20 minutes at 20 to 25°C, 5m1 of sodium hydroxide was added. Absorbance was taken after 5 minutes at 546nm using spectrophotometer and the result of the test was read of from AST standard curve.

Determination of Alanine Amino Transferase Activity (Alt).

The enzyme ALT catalyses the transfer of amino group form L- alanine to aoxoglutamate and pyruvate.

Method:

The alanine transferase activity was determined using the method specified in the randox kits.

Principle:

a- oxoglutarate + L- alanine - L- glutamate + pyruvate. ALT activity is measured by monitpring the concentration of pyruvate hydazone formed with 2,4-dinitrophenylhydrazine.

Table 2 Reagent composition

Reagent 1	Contents	Initial concentration of sodium.
	Buffer; phosphate buffer	(l00mmmol/L, PH 7.4)
	L-aspartate	(l00mmol/L)
	A- Ketoglutarate	(2mmol/L)
Reagent 2	2,4- dinitrophenyl-hydrazine	(2mmol/L)

Procedure:

0.1ml of the sample was pipette into test-tubes, 0.5 ml of reagent 1 was added and mixed. The mixture was incubated for 30 minutes at 37°C. Reagent 2was added and solution was allowed to stand for 20 minutes at 20 to 25°C, 5m1 of sodium hydroxide was added. Absorbance was taken after 5 minutes at 546nm using spectrophotometer and the result of the test was read of from AST standard curve.

Determination of Alanine Amino Transferase Activity(ALT).

The enzyme ALT catalyses the transfer of amino group form L- alanine to aoxoglutamate and pyruvate.

Method:

The alanine transferase activity was determined using the method specified in the randox kits.

Principle:

a- oxoglutarate + L- alanine - L- glutamate + pyruvate. ALT activity is measured by monitoring the concentration of pyruvate hydazone formed with 2, 4-dinitrophenylhydrazine.

Result

Table 3: Effect of bitter leaf on the activities of hepatic enzymes in wistar albino rats fed crude oil contaminated diet

CAGE A	CAGE B	CAGE C	CAGE D	CAGE E	CAGE F
AST(U/g) 48.98±1.587" 53.47±1 .03a	56.05±0.57a	69.94±1.61b	74.17±10.35b	87.10±7.77b	
ALT (U/g) 31 .62±4.94a	40.96±1.03a	56.05±0.57a	45.00±121b	74.17±10.35b	78.09±4.65b
ALP (U/g) 112.63±0.83a	126.77±2.62a	130.94±1.21a	124.01±48.18a	172.01±5.51b	186.56±5.48b
ACP (UIg) 4.48±0.36a	6.87±0.56a	8.07±0.88a	1188061b	17.99±1.62b	20.82±1,27b

Results are expressed in mean ± standard deviation. Values sharing different superscript across a row differ significantly. The mean difference is significant at the P <0.05 1vel, n = 4 for all group.
Where, A= Control; B= feed + 5g of bitter leaf; C= feed+l0g of bitter leaf; D feed+5g of bitter leaf+ 4m1 of crude oil; E feed+ l0g of bitter leaf+ 4m1 of crude oil; F= feed + 4m1 of crude oil.
Table 3 shows a significant increase in the activities of AST, ALT, ALP and ACP in rats fed crude oil contaminated diet as compared to the values obtained from the control. However, supplementation of crude oil contaminated diet with bitter leaf extracts significantly decreased the activities of AST, ALT, ALP and ACP compared to control.

Discussion

Crude oil has been reported to induce a myriad of biochemical effect in animal which includes hepatoxicity (Achuba and Ogumu, 2014ab, Achuba and Nwokogba, 2015ab). In previous reports crude oil have been implicated in inducing liver damage. This is in agreement with this study (Table 3), in which there is usually increase in the liver marker enzymes, AST,ALP,ACP and ALT in the serum of rats fed with crude oil contaminated diet.

The increased levels of ALT and AST indicate hepatic damage as these cytoplasmic marker enzymes are released into circulation after cellular damage (Lin *et al*.,2000; Patrick- Iwuanyanwu*et al.,* 2011, Momoh and Damazio, 2014).

In this current study in cooperation of bitter leaf into the crude oil contaminated diet reduced the serum level of these marker enzymes relative to the values in that fed with crude oil contaminated diet. This agrees with earlier reports in which Petroleum hydrocarbon induced hepatological damage was earlier reported to be mitigated by palm oil in the diet (Achuba and Ogumu, 2014). It might be possible that the bitter leaf was able to reduce crude oil hepatoxicity which may

be attributed to. its antioxidant potential, this in line with the protection exhibited by palm oil has shown that antioxidants may induce their protection by reducing the susceptibility of liver cells to the damaging effects of free radicals generated from the metabolism of aliphatic and aromatic hydrocarbons present in crude oil. As the Protective effect of antioxidants has been previously reported (Achuba 2005, Ubohe*t al.,*2009a; 2009b; 2009c).

Conclusion

The present study showed that the consumption of crude oil contaminated diet may result in damage liver damage. However, the consumption of bitter leaf may offer some protection against crude oil induced hepatoxicity.

It is hereby recommended that:

A longer period of study should be carried out to find the long-term effects of exposure;

Both lower and higher percentage concentrations of both bitter leaf and crude oil should also be studied over a long period of exposure.

References

Achuba, F.I. (2005). Effect of vitamins C and E int1ce on blood lipid concentration, lipid peroxidation, Superoxide dismutase and catalase activities in rabbit fed petroleum contaminated diet. Pakistan Journal of Nutrition 4(5): 330-335.

Achuba, F.I. and Osakwe, S.A. (2003). Petroleum induced free radical toxicity in African catfish (Clariagariepinus). Fish Physiol. Biochem., 29: 97—103.

Adeola,'. 0., 1996. Bioavailability of tryptophan in soybean meal FOR 10kg pigs using slope ratio assay. J. Anim. Sci., 74(10); 2411-2419

Amira CA, Okubadejo NU (2007) Frequency of complementary and alternative medicine utilization in hypertensive patients attending an urban tertiary care centres in Nigeria. BMC Compl. Alternative Med. 7:30—48.

Breimer, L. H. (1990). Molecular mechanisms of oxygen radical carcinogenesis and mutagenesis: The role of DNA base damage. Mol. Carcinogenesis, 3: 188—197.

Egborge, A.B.M. (1991). Industrialization and heavy metal pollution in Warn River. 32nd Inaugural Lecture, University of Benin, Benin City, Nigeria

Ekweozor. I.K.E. (1989). A review of the effects of oil pollution in West African Environment. Discovery cnd Innovation 1(3): 27-37.

Halliwell B, Rafter J, Jenner A (2005) Health promotion by flavonoids, tocopherols,tocotrienols, and other phenols: Direct or indirect effect? Antioxidant or not? The Am. J. Clin. Nutria. 81: 268S-276S.

Huffman MA (2001) Self-medicative behavior in the African great apes: An evolutionary perspective into the origins of human traditional medicine. Boisei. 51:651—661.

Huffman MA, Koshimizu K, Ohigashi H (1996) Ethnobotany and zoopharmacognosy of Vemoniaamygdalina, a medicinal plant used by humans and chimpanzees. Biol. Utilization. 2: 351-360.

Huffmn MA, Seifu M (1989) Observations on illness and consumption of a possibly medicinal plant Vernoniaamygdalina (Del.), by a wild Chimpanzee in the Mahale Mountains National parks Tanzania. In: Primates 30 : 5 1-63.

Huffrnan, M.A., Seifu, M. (1989): Observations of the illness and composition of a medicinal plant vernoniaamygdalina by wild chimpanzee in the mohale mountains, Tanzania primates.

Iruonagbe, T. C. (2008). The Niger Delta crisis: challenges and prospects for peace and stability. Int'l conf on the Nigerian state oil industry and the Niger Delta. Harey pub. Pp 633-642.

Jessup, D.A. and Leightion, F.A. (1996). Oil pollution and petroleum toxicity to wildlife. In:
Non-infectious disease of wildlife. 2nd ed (fair broshter, A., Locke, L.N. 'and Holf, G.L. (eds)). Iowa State University Press. Pp 141—156.

Jisaka M, Ohigashi H, Takegawa K, Hirota M, Irie R et al. (1993) Steroid glucosides from Vernoniaamygdalina, a possible chimpanzee medicinal plant. Phytochemistry 34:409—413.

Jisaka m, ohigashi h, takegawa k, Huffman ma, koshimizuk(1993). "antitumoral and antimicrobial activities of bitter sesquiterpelielatones of vernoniaamygdalina, a possible medical agent used by wild chimpanzees". Bioscibiochem. 57 (5);833.

Katwisak Van M:'M, Schmitz G.H.W, Gasseling AP, Avesaath and P.1-I. Van (1999). Effects of salinity and nutrient load and their interaction on zostera marina. Mar. Ecol. Prog. Sen 190.155-165.

Khan, A.A., Coppock, R.W. and Schuler, M. Ivi. (2001). Effect of multiple exposure of small dose of Pembina cardium crude oil and diesel in rat. Arch. Environ. Contam. Toxicol.,40: 418—424.

Koshimizu K, Ohigashi H, Huffman MA (1994) Use of Vernoniaamygdalina by wild chimpanzee: possible roles of its bitter and related constituents. PhysiolBehav 56:1209-1216
Olsen, David M, Eric dinerstein, Neil G. Burgess BioSci (2001) 51 (1 1):933-938.https://doi.orgllO.1641/0006-3568(2001)051

Koshimizu 'K, Ohigashi H, Huffman MA (1994) Use of Vernoniaamygdalina by wild

chimpanzee: possible roles of its bitter and related constituents. PhysiolBehav 56: 1209-1216.

Lin, S.C, Chung, T.C. T.H. Ueng, Y.H. Linn, S.H. Hsu, C.L. Chiang and Lu F, Foo LY (2001) Antioxidant activities of polyphenols from sage (Saliviaofficinalis). Food Chem. 75: 197-202

Momoh Johnson and Damazio .O.A.(2014) Hepatotoxicity of House Hold Kerosene (HHK) on Liver Enzyme Markers and Its Effect on Hematological and Oxidative Stress Parameters on Wistar Albino Rats Volume 2014, ISSN: 2276-7487.

Patrick-Iwuanyanwu, K.C. Onyemaenu, C.C., Wegwu, M.O and Ayalogu, E.O (2011). Hapatotoxic and nephrotoxic Effect of kerosene and petrol-contaminated diet in the wistar albino rats. Research Journal of Environmental Toxicology 5:49-57

Rornero, F.J., Bosch-Morell, F., Romero, M.J., Jareno, E.J. and Marine, N. (1998). Lipid peroxidation products and antioxidant in human disease. Environ. Health Perspect.,106(5): 1390—1393

Souza, M.' F., Tome, A.R. and Rao, V.S. (1999). Ihhibition by the flavonoid ternatin on aflatoxin B 1-induced lipid peroxidation in rat liver. J. Pharm. Pharmacol., 51: 125—129.

Uchenna VO, Chinwe EO, John MO, Ijeoma OE (2008)Hypoglycemic indices of Vernoniaamygdalina on postprandial blood glucose concentration of halthy humans. Afr J Biotechnol. 7: 4581—4585.

Udosen EQ (1995) Proximate and mineral composition of some Nigerian vegetables. Discov. Nino. 7: 383-386.

Val, A.L. and Almeida-Val, V.M.F. (1999). Effects of crude oil on respiratory aspect of some fish species of the amazon. In: Biology of Tropical Fish, Manaus brasil. pp 227—291.