GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 12 NO. 4 2006 : 501 - 504
COPYRIGHT© BACHUDO SCIENCE CO. LTD PRINTED IN NIGERIA. ISSN 1118 - 0579

EFFECT OF DIETS CONTAMINATED WITH CRUDE PETROLEUM PRODUCTS (BONNYLIGHT AND FORCADOS) ON ENZYME ACTIVITIES OF WISTAR ALBINO RATS

J. O. AKANINWOR and E. A. OKEKE

(Received 16 May, 2006; Revision Accepted 20 July, 2006)

ABSTRACT

Changes in enzyme activities (ALT, ALP and AST) in Wistar Albino rats chronically exposed to diets contaminated with crude petroleum samples (Bonnylight and Forcados) have been investigated. Increases in the activities of the enzymes (ALP, ALT and AST µ/l) in the test animals over the control were observed. ALP: (male rats) Control 57.50±2.08< Bonnylight 90.50± 17.37 and Forcados 81.33±7.02; (female rat) Control 55.75 ± 7.14< Bonnylight 89.25±17.39 and Forcados 95.75 ±4.86.
ALT: (male rats) Control 45.50±2.65 < Bonnylight 120.00 ± 39.99 and Forcados 112. 67± 9.29; (female rats) Control 46.00± 9.56 < Bonnylight 101.75 ± 13.30 and Forcados 131.25 ± 7.14
AST: (male rats) Control 48.00± 2.58 < Bonnylight 125.00 ± 44.97 and Forcados 120.67 ± 2.08; (female rats) Control 46.50 ± 7.33 < Bonnylight 105.50 ± 13.96 and Forcados 130.75 ± 14.69.
These increased levels of liver enzyme activities in the test animals over the control are indicative of injuries / damages to the liver by toxic metabolites produced by the crude petroleum samples which would cause dysfunction and might eventually be the cause of death of some of the test animals as observed. A significant rise in AST is implicated in myocardial infarction while increases in ALT and ALP is implicated in hepatocellular damage suggesting that these petroleum crude samples (Bonnylight and Forcados) on long term exposure to animals or even humans that feed on these animals in an environment polluted with them will cause deleterious effects on the liver of such and may reduce the population around these areas. A close monitor / treatment of the levels of xenobiotics accruing from effluent discharge or total spillage from oil companies is necesasited to maintain safety levels of the environment.

KEY WORDS: *Enzyme bonnylight, forcados pollution* and environment

INTRODUCTION

Crude petroleum is made of mixtures of various organic substances which are sometimes difficult to separate. The organic substance are primarily and principally compounds comprising of carbon and hydrogen, otherwise referred to as HYDROCARBONS. Sulphur, oxygen and nitrogen are also present in crude oil (Payne et al, 1983). Organometals such as Vanadium, iron, copper and nickel are found in petroleum crude oil in low concentrations (Aksoy, 1985). Most of the substances contained in crude petroleum occur naturally due to their presence in rock formation or in salt water deposits from which the crude oil was drawn (Annon, 1973). However, some of these metals are introduced from the drilling pipes and drilling fluid additives while others are introduced during pumping, preparing and transporting of crude oil (IARC, 1989) greatly in their relative concentrations of different components and thus show substantial variability in solubility, dispensability, persistence and toxicity (Anderson et al, 1974). These toxicants are suspected to have the potential and capacity of affecting different organs in the body in situations of occupational exposure of workers in the oil sector or exposure of plants, animals and humans in oil- spill environment. The primary targets of toxicity could include the lungs, liver, kidney, neuroendocrine system, testes and post testicular sites (Sherman and Sherman, 1979). The uniqueness of crude petroleum is such that two samples from different accumulations could be matched in composition or properties (IARC, 1989). The hydrocarbon, sulphur, oxygen, nitrogen and organometals that make up Nigerian crude petroleum are obtained from decomposition of aquatic substances such as marine animals and plants which have been bunied under various layers of mud as silt (Awobajo,1981). They have also been grouped into types as light, medium (intermediate) and heavy depending on their density and physical and chemical properties.

Offshore pollution of environment by crude petroleum spills may result during loading and unloading operations of tankers, crude oil storage tanks, pipeline and leaky badges causing accidental release of oil (Kuhnhold, 1980). Also, spillage can result from break up of tankers, clearing and flushing of oil tanks at sea leading to contamination of various areas of shoreline and soil. Many beaches have been affected by this type of pollution especially those located near shipping points and large refineries (Shaheen, 1992). Crude Petroleum when spilled has a potential of creating drifting nuisance and has the capacity of covering large expanses of water in relative short time. There is therefore no doubt that crude oil inflicts damages to the surface, marine and coastal ecosystem on a long-term exposure (Moore and Dwyer, 1974, Ezeala, 1987).

A usual analysis of mammalian tissues such as the liver, heart, kidney etc involves enzymes amongst which are the Alkaline Phosphatase (ALP), Alanine Amino Transferase (ALT) or Glutamate Pryruvate Transaminase (GPT) and Aspartate Amino Transaminase (AST) or Ghtamate Oxaloacetate Transminase (GOT). These enzymes are naturally occurring macromolecules which catalyze the biochemical reactions of the body (Richen and Paumgartner, 1980); they do this by speeding up reactions that might otherwise proceed very slowly. Each tissue has its own specific enzymes, with one enzyme being common to more than one type of tissue (Bouchair, 1980). The enzyme, Alkaline Phosphatase (ALP) is found mainly in the bone, liver, small amounts in kidney and the gastrointestinal tracts. While AST or GOT is found mainly in the heart, skeletal muscles, liver, kidney and the red blood cells; ALT is relatively higher in the liver than in the myocardium; it is also

J. O. Akaninwor, Department of Biochemistry, University of Port-Harcourt, Port-Harcourt, Nigeria.
E. A. Okeke, Department of Biochemistry, University of Port-Harcourt, Port-Harcourt, Nigeria.

present in a cytoplasm in a parenchyma cells (Sherlock, 1951). The elevation of these enzymes implies that a particular tissue is damaged enough to release significant quantities of the enzymes into the blood stream (Farber and Fisher, 1979). Abnormal levels of these enzymes are implicated in disease condition such as myocardial infarction, viral hepatitis, jaundice, cirrhosis of the liver and various types of bone diseases (Davidson, 1979). The liver has a valuable role in the detoxification of many substances and there is an indication that endogenous substances undergo hepatic conjugation to facilitate the excretion of the exogenous toxic compounds (Patrick and McGee, 1988). Also, it contains numerous enzymes some of which are present in serum in very low concentration. These enzymes have no known functions in the serum other than to provide information about hepatic state and disorders. It has been reported that elevated serum transferase and alkaline phosphate levels are indicators of liver necrosis. This study has tried to investigate the activities of the enzymes in rats fed diets contaminated with petroleum products.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS:

Wistar Albino rats were obtained from the Animal House of the Department of Biochemistry, University of Port-Harcourt, Port Harcourt, Nigeria. The animals were acclimatized for one week in metabolic cages of the animal house before the commencement of the experiment.

CRUDE PETROLEUM PRODUCTS AND THEIR SOURCES:

The crude petroleum sample (Bonnylight and Forcados) types were obtained from the Nigerian National Petroleum Corporation (N.N.PC.) zonal office at Moscow Road, Port Harcourt, precisely from their Quality Control Analytical research laboratory and stored in two 4 liters industrial bottles at room temperature to avoid reaction with light.

CHRONIC TOXICITY TEST (FEEDING STUDY):

The chronic toxicity test involved subjecting the experimental animals to a feeding regimen for 90 days. Three different diets were prepared which were molded into pellets with Bonnylight and Forcados crude petroleum.

The animals were divided into three groups of 8 rats per group Bonnylight (4 males and 4 females), Forcados (4 males and 4 females) and control (4 males and 4 females). The rats were between ages 60-80 days old and were weighing between 140g and 200g. The weights of the rats in each group were harmonized after which the animals were placed in individual metabolic cages (one rat per cage). The rats were acclimatized for seven days followed by the commencement of feeding exercise on diets, which were prepared with 27.08g/kg of Bonnylight crude petroleum for the Bonnylight group and 37.68g/kg of Forcados crude petroleum for the Forcados group. The control diet was prepared with normal saline for the control group. The animals were fed ad libitum for 90 days during which their drinking water was changed and the cages cleaned daily. The rats sacrificed at the end of the 90 days feeding period and analysis carried out on their tissues.

The actual toxicity test was carried out with 50 weaning Wistar Albino rats with body weights between 100g and 299g. The body weights were harmonized and the rats placed in 5 groups of 5 rats each with varying dose levels of Bonnylight and Forcados crude petroleum administered to the rats by injection, intraperitoneally. The sexes of the animals were disregarded in the exercise. Rats in group one of each test sample were administered with 10mls of normal saline (control) while the rest of the group (2to5) were injected 10mls, 20mls, 30mls and 60mls of each of the test samples respectively. Observations were made after 24 hours of the experiment.

BLOOD PREPARTION

Blood was collected into sample bottles with anticoagulant (heparin) from the cardiac regions of the test animals and the control group.

METHODS

BIOCHEMICAL ASSAY OF EMZYMES:

The enzyme analyzed included the Alkaline Phosphatase (ALP), Alanine Amino Transferase (ALT) or Glutamate Pryruvate Transaminase (GPT) and Aspartate Transaminase or Glutamate Oxaloacetate Transaminase (GOT). The analyses of these enzymes were carried out using the Reflotron System equipment. This was turned on and 1ml of whole blood sample drawn into reflotron pipette then applied on the specific enzyme test strip. The test strip containing the blood sample was inserted into the reflotron equipment via the test pad. Reading was determined photometrically at 567nm and results displayed after 120 sec. This method was used for the three enzymes under study.

RESULTS AND DISCUSSION

RESULTS

EFFECT OF CHRONIC INTAKE OF DIETS CONTAMINATED WITH CRUDE PETROLEUM ON ENZYMES

Levels of ALP, ALT and AST of rats in the two test groups of Bonnylight and Forcados were observed to be much higher than those of the control group. (Table 1)

TABLE 1: ENZYME ACTITIVIES OF ALP, ALT (GPT) AND AST (GOT) IN RATS FED BONNYLIGHT AND FORCADOS CRUDE PETROLEUM CONTAMINATION DIETS.

	MALE RATS			FEMALE RATS		
ENZYMES	CONTROL (NORMAL SALINE)	BONNYLIGHT	FORCADOS	CONTROL (NORMAL SALINE)	BONNYLIGH	FORCADOS
	0.00	27.08 g/kg	37.68 g/kg	0.00	27.08 g/kg	37.68 g/kg
ALP	$57.50^{a} \pm 2.08$	$90.50^{a} \pm 17.37$	$81.33^{b} \pm 7.02$	$55.75^{a} \pm 7.14$	$89.25^{b} \pm 17.39$	$96.75^{b} \pm 4.86$
ALT (GPT)	$45.50^{a} \pm 2.65$	$120.00^{b} \pm 39.99$	$112.67^{b} \pm 9.29$	$46.00^{a} \pm 9.56$	$101.75^{b} \pm 13.30$	$131.25^{c} \pm 7.14$
AST (GOT)	$48.00^{a} \pm 2.58$	$125.00^{b} \pm 44.97$	$120.67^{b} \pm 2.08$	$46.00^{a} \pm 7.33$	$106.50^{b} \pm 13.96$	$130.75^{b} \pm 14.59$

a,,b,c, - Mean ± S.D, not followed by the same alphabet are significantly different at 5% ($P < 0.05$) level of probability based on Duncan multiple range test.

There was no significant difference ($p>0.05$) in the levels of all the enzymes studied in 15 rats of the Bonnylight with Forcados crude. Also there was no significant difference ($p>0.05$) in the enzymes levels in both male and female rats for either the groups on Bonnylight or Forcados crude petroleum contaminated diets.

ALP; (male rats) control 57.50 ± 20.08 < Bonnylight 90.50 ± 17.37 and Forcados 81.33 ± 7.02 ; (female rats) Control 55.57 ±7.14< Bonnylight 89.25 ± 17.39 and Forcados 95.75 ± 4.86.

ALT: (male rats) Control 45.50± 2.65 < Bonnylight 120 ± 39.99 and Forcados 112.67 ±9.29; (female rats) Control 46.00 + 9.56 < Bonnylight 101.75 ± 13.30 and Forcados 131.25 ± 7.14

AST: (male rats) Control 48.00 ± 2.58 < Bonnylight 125.00 ± 44.97 and Forcados 120.67±2.08; (female rats) Control 46.50 ± 7.33 < Bonnylight 106.50 ± 13.96 and Forcados 130 .75 ± 14.59.

ACUTE TOXICITY OF BONNYLIGHT AND FORCADOS CRUDE PETROLEUM PRODUCTS ON MORTALITY OF RATS

These are shown in Tables 2 and 3

In both cases of the test diets, the mortality rates increased with increased dosages or contamination of the diets over the control. A dose of 254.80g/kg and 354.60g/kg (for Bonnylight and Forcados respectively) were able to bring about total death of the number of rats used indicating high toxicity of these crude petroleum products.

TABLE 2: MORTALITY RATE OF RATS TREATED WITH BONNYLIGHT CRUDE PETROLEUM PRODUCTS

GROUP	DOSE g/kg	No of death	No alive	Average time of death (hrs)
1	0	0	5	-
2	63.7	2	3	18
3	127.4	3	2	14
4	191.1	4	1	10
5	254.8	5	0	5

n = 5

LD_{50} (g/kg) = 108.30

TABLE 3:MORTALITY RATE OF RATS TREATED WITH FORCADOS CRUDE PETROLEUM PRODUCTS

GROUP	DOSE g/kg	No of death	No alive	Average time of death (hrs)
1	0	0	5	-
2	88.70	2	3	20
3	177.30	3	2	12
4	266.00	4	1	9
5	354.60	5	0	6

n = 5 rats

LD_{50} (g/kg) = 150.70

DISCUSSION

Results from Table 1 showed significant increase ($P<0.05$) in the activity of ALP, ALT (GPT) and AST (GOT) in rats of the Bonnylight and Forcados groups compared with the rats of the control group. However, the results indicated that there was no significant difference ($p>0.05$) in the serum levels of these enzymes between the male and female rats in the test group.

On the other hand, there were significant increases ($P<0.05$) in the levels of the enzyme in the male rats of Bonnylight group when compared with the control. Also, the male rats of the Forcados group increased significantly ($P<0.05$) in their levels of the enzymes when compared with the control group but there was no significant difference ($P>0.05$) in the enzyme levels of male rats of Bonnylight group in comparison with those of the Forcados group; even as the male rats of the Bonnylight group showed slightly higher levels of the enzymes.

The female rats of the Bonnylight group showed significant increases ($P<0.05$) in their levels of the enzymes when compared with the control group. Also the female rats of the Forcados group increased significantly in their levels of the enzymes when compared with the control group but there was no significant difference in the enzymes of the female rats of Bonnylight and Forcados groups; even as the female rats of the Forcados group showed slightly higher levels of the enzymes than those of Bonnylight group.

Kaplowitz (1992) had indicated that elevated Aspartate Transferase (AST) and Alkaline Phosphates (ALP) levels are indicators of liver damage while

ALT activity may reflect some inflammatory diseases or injury to the liver. Dheer et al, (1987) and Sharpe et al (1996) have illustrated that enzyme pattern in the serum; reflects the physiological state of the organs and tissues. For instance, increases in levels of ALP, ALT and AST were observed in the serum of fish exposed to 2,3,4, triaminazobenzene which resulted in hepatocellular damage (Krishna and Veena, 1980).

Other studies also indicated increases in the activities of the liver enzymes following liver damage in fish and albino mouse exposed to toxic substances (Dheer et al, 1987, Sharpe et al, 1996 and Mohssen, 1997).

These reports and the report of Dixon and Dixon (1976), which observed increases in the levels of enzyme activity in fish from area near oil installations than those from clear zones, support the findings in this work.

The serum levels of these enzymes reflect the physiological state of the liver and the levels change according to distortion of the liver, resulting from cellular injury of the organ caused by toxic metabolites and diseases. These must be reason for high mortality rates observed (table 3 and 4) during the acute toxicity assay.

SUMMARY AND CONCLUSION

This study carried out on possible deleterious effects of the crude petroleum products on enzyme activities of Wistar Albino rats has shown increases in the activities of the enzymes (ALP, ALT and AST μ/L) in the first animal over the control irrespective of the sex of the animals. Liver being an important organ of the rats and even humans on exposure to these xenobiotics arising from contact with crude petroleum products (Bonnylight and Forcados) was severely damaged in earlier work. (Akaninwor and Okeke in press). This damage was reflected in the high levels of the blood enzymes and even death of the rats. It thus becomes imperative that constant and prolonged exposure of life in both occupational and natural existence will reduce life span of the populace concerned. It is therefore important that regular monitoring via analyses of industrial metabolites and

effluents into surrounding vicinities inhabited by flora, fauna and humans must be employed by the industries concerned and Government in a bid to maintaining safe levels of this toxicants.

REFERENCES

Akaninwor, J. O. and Okeke E. A. (In press). Effect of Diet Contaminated with Crude Petroleum Products (Bonnylight and Forcados) on Hormones and Histopathological parameters of Wistar Albino Rat.

Aksoy, M., 1985. Malignancies due to Occupational Exposure to Benzene. Am. J. Ind. Med., 7:395 – 402.

Anderson, J, W., Neff, B., Acox, H. E., Talem, H. E, and Hightower, G. M., 1974. Characteristics of Dispersions and Water Soluble Extracts of Crude and Refined Oils and their Toxicity to Estuarine Crustaceans and Fish. Biol. 27:74-88.

Annon, O., 1973. Evaluation of World's Important Crude. The Petroleum Publishing Company; Oklahoma, U.S.A. 60.

Awobajo, A.O., 1981. An analysis of Oil Spill Incidents in Nigeria. Proceeding of National Seminar on Petroleum Industries and Nigeria Environment, Warri; 57-63.

Bouchair, I.A.D., 1980. The Medical Treatment of Gallstones. Annu. Rev. Med., 31:59.

Davidson C.S., 1979. Problems in Liver Diseases. Stratton Intercontinental Medical Book Corps New York 223-225.

Dheer J. M. S., Dheer T.R. and Mahajan, C.L., 1987. Haematological and Haematopiotic Response to Acid Stress in an breathing Fresh Water Fish. Channa Punctatus, Bioch J. fish Biol. 30:577-588.

Dixon T. J. and Dixon T. R., 1976. Microbial Degradation of Industrial Chemicals. In: Michael, R. eds. Environment Microbiology; Wiley, New York 2: 150-180.

Ezeala D.O., 1987. The Sensitivity of Pista Stratoites (a fresh water plant) to Crude Oil Pollution. Tropic Agric, 28:194-196.

IARC, 1989. IRAC Monographs on the Evaluation of the Carcinogenic risk of Chemical to Humans: Some Industrial Chemicals and Dyestuffs. Lyon; 29: 93, 148, 391-398.

Kaplowitz N., 1992. Liver and Billary Diseases. Williams and Wilkins, London. 3.

Krishan, A. G. and Veen, G., 1980. 2, 4, 4-Triminoazo benzene-induced Haematobiochemical Anomalies in Fish (Chanma Puntatus Bull). Environ. Contam. Toxicol. 25: 136-141.

Kuhnhold W. W. Everish, D., Stegeman, J. T. Lake J and Woike, R.E., 1980. Effects of Low Level hydrocarbon on Embryonic, Larval and Adult winter flounder (pseudopleurnertes americanus): Impact of Oil Spills. 14-17 June, 1978, Keystans, Colorada, USA. American Institute of Biological Science, Washington, D.C., 678-680.

Mohssen, M., 1997. Inhalation toxicity studies of theme (phonate) in male Swiss Albino mouse: MUS MUSCULUS: Environment pollution. 96 (3) : 383-288.

Moore, S.F. and Dwyer R.L., 1974. Effects of oil on marine organisms: A critical Assessment of Published data on water resources 8:819-827.

Payne, J. F. Kiceniuk, J and Misra, R., 1983. Sublethal Effects of Petroleum Hydrocarbons on Adults American Lobsters (Homarus americanus); Can. J. Fish. Aquat Sci.; 40: 705-717.

Reichen J. and Pumgartner, 1980. Excretory Functions of the liver. In: Javitt, N.B. ed. International Review of physiology; liver and Billiary Tract Physiology. University Park Press, Baltimore. 25-29.

Shaheen I. E., 1992. Technology of Environment Pollution Control. Penn well Publishing Company, U.S.A. 226-301.

Sharpe P.C., Mc Bride R. and Archbold, G.P, 1996. Biochemical Markers of Alcohol Abuse. Medicine, 89(2) :137-144.

Sherlock, S., 1951. Liver Disease. Churchchill, London 71 – 73.

Sherman, I. W. and Sherman V. G., 1979. Bology: A Human Approach. Oxford University Press, New York 153-154.