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Interference of Bony Light Crude Oil (BLCO) Contaminated Feed on Cellular Status and Oxidative Stress Markers in Rat's Heart Homogenates.

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ABSTRACT

The impact of crude oil mixed meal on cellular status and oxidative stress markers in rat's heart homogenates was studied. 35 Wistar rats of similar weight were randomly divided into 7 groups as follows; Group 1 control (normal chow), Group 2 (Treated with 3.88g/kg crude oil mixed meal), Group 3 (Treated with 7.75g/kg crude oil mixed meal), Group 4 (Treated with 15.51g/kg crude oil mixed meal), Group 5 (Treated with 32.01g/kg crude oil mixed meal), Group 6 (Treated with 62.02g/kg crude oil mixed meal), and Group 7 (myocardial infarct-induced group). Treatments in various groups were administered for 8 weeks (exposure period) and were later withdrawn for 2 weeks (withdrawal period). 5 ml of blood was taken from all groups via cardiac puncture in both phases for analysis for electrolytes estimations, haematological parameters, lipid profiles, and liver enzyme assay. Heart tissues were taken and homogenized and prepared for cardiac oxidative stress markers analysis, were extrapolated and calculated. Results from various laboratory analyses were statistically analysed using ANOVA (SPSS) and presented in tables and charts with level of significance at $P \leq 0.05$. Haematological indices, electrolytes liver enzymes profile, lipid parameters and oxidative stress markers all presented marked increase during the exposure phase of six weeks. In the withdrawal phase, virtually all the above measured parameters were reversed and the corresponding biological effects ameliorated. The implications of the above extrapolates in both phases indicated that crude oil exposure could trigger, electrolyte imbalance, cellular disruptions, liver assault, and can be highly detrimental and delirious to cells while withdrawal from the contaminated meal was observed to reversed the entire scenarios. In conclusion, crude oil contaminated feed on cardiovascular integrity and risk factors could be a huge challenge and a potent pre-disposing scenario to various debilitating diseases of the heart on prolonged exposure.

Keywords: crude oil mixed meal, electrolytes, oxidative stress markers, liver enzymes, lipids.

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INTRODUCTION

Crude oil can interrupt a key pathway found in the hearts of fish and other marine animals. The 2010 Deepwater Horizon oil spill exposed tuna and several marine organisms to high levels of crude oil (Steven *et al*., 2014)

There are reported attempts to find the specific cause for the elevated heart problems in fish near oil spills and to explain why humans exposed to air pollution have increased risk of heart attacks (Steven et al., 2014)

Exposure to oil has previously been shown to have physiological consequences to the heart, and can cause deformations and death in eggs and larval fish, making it crucial to understand the effects in order to assess the impacts of oil spills (Steven et al., 2014)

The ingestion of petroleum has been reported to induce biological stress (Val and AlmeidaVal, 1999) 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, 1994) that damages critical cellular macromolecules such as DNA, lipids and proteins (Breimer, 1990; Romero et al., 1998; Souza *et al*., 1999) and results in inactivation of antioxidant enzymes (Pigeolet *et al*., 1990). Biochemical biomarkers are increasingly being used in ecological risk assessment of the ecosystem to identify the incidence of exposure and effects caused by xenobiotics (Olsen *et al*., 2001). The most powerful tools for the investigation of pollutants ―*in situ*‖ are biomarkers. Ideally, biomarkers will identify effects at sub-cellular level before they manifest at higher levels of biological organization (McCarthy and Shugart, 1990). The use of biomarkers in environmental monitoring is now becoming a routine method for examining toxicity of chemicals (Onwurah, 1999; Shertzer *et al*., 1994)

MATERIALS AND METHOD

Animal Preparation

All animals were obtained from the animal house, faculty of Basic Medical Science, University of Port Harcourt, Nigeria. Albino wistar rats weighing 120-150g were housed in wooden cages for at least two weeks in the animal room and allowed to acclimatize for 4 weeks.

Crude Oil

Crude oil **of** BLCO variant was obtained from the Nigerian National Petroleum Corporation (NNPC) Warri, Nigeria. The crude oil was diluted in olive oil according to previous studies (Dede et al., 2002; Owu et al., 2005) and then mixed thoroughly with the animal meal.

Administration of crude oil

A weighed quantity of crude oil was mixed with a weighed quantity of normal rat chow and served to the animals in various test groups on a daily basis for 8 weeks. The daily ration of the crude oil mixed meal was prepared afresh each day of the study.

Grouping Design and Treatment

35 Wistar rats of similar weight were randomly divided into 7 groups as follows; Group 1 control (normal chow), Group 2 (Treated with 3.88g/kg crude oil mixed meal), Group 3 (Treated with 7.75g/kg crude oil mixed meal), Group 4 (Treated with 15.51g/kg crude oil mixed meal), Group 5 (Treated with 32.01g/kg crude oil mixed meal)

Collection of blood and serum

Ten to fifteen (10-15) drops of blood (< 1ml) were obtained from each rat into sample bottles containing 10% ethylene diamine tetra acetate (EDTA) as anticoagulant. The blood was gently rocked and used for haematological studies and for the quantification of the parasites. Blood for serum was obtained by collecting 40-50 drops of blood (> 2mls) into sample bottles into which no anticoagulant was added. The bottles were placed in slanting positions for 2-3 hours to allow the blood to clot and yield serum. The clot was removed and the serum centrifuged for 5 minutes at 5, 000 rpm. The serum was then gently decanted into eppendorf tubes, stored at -20OC until used for the assays of haematological parameters, electrolytes, Urea and creatinine, liver enzymes.

PCV, Total WBC and Differential WBC counts

The general procedures for Veterinary Haematology and Practical Haematology as described by Schalm (1965) and Coles (1986) respectively were adopted in the determination of the packed cell volume (PCV), total white blood cell (TWBC) and differential white blood cell (DWBC) counts.

Serum Urea & Creatinine

Determination of serum creatinine was based on the modified Jaffe method (Fossati et al., 1983) for in vitro determination of creatinine in serum, plasma or urine using Randox creatinine colorimetric method with depolarisation (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

Serum Alkaline phosphatase

Determination of serum alkaline phosphatase was done using the phenolphthalein monophosphate method (Klein et al., 1960) for the in vitro determination of alkaline phosphatase in serum or plasma using Quimica Clinica test kit (QCA, CN-340km 1081- P.O. BOX 20-E43870 AMPOSTA/Spain).

Serum urea

Determination of serum urea was done using Urease Berthelot method (Fawcett et al., 1960) for quantitative in vitro determination of urea in serum, plasma and urine using Randox urea colorimetric kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

Serum aspartate aminotransferase (AST)

Serum aspartate aminotransferase was determined using the standard colorimetric method of Reitman and Frankel (1957). This is an in vitro method of determining AST using Randox Glutamic-oxaloacetic transaminase test kit (Randox Laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

Serum alanine aminotransferase (ALT)

Serum alanine aminotransferase was determined using the standard colorimetric method of Reitman and Frankel (1957). This is an in vitro method of determining ALT in serum using 50 Randox Glutamic-pyruvic transaminase test kit (Randox laboratories Ltd. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom, BT29 4QY).

Determination of total proteins

The total proteins was determined using the method of Lowry (1951).

Oxidative Stress Markers Analysis

Preparation of Tissue Homogenate

- The heart was immediately removed, rinsed with KCl and weighed. The heart was then be homogenized in four volumes of the homogenizing buffer (0.1M Tris-KCl, pH 7.4) using a Teflon homogenizer.
- The resulting homogenate was centrifuged at 12,500 g for 15 minutes in a cold centrifuge (4°C) to obtain the post mitochondrial fraction. The supernatant was collected and used for oxidative stress markers analyses.

Determination of superoxide dismutase (SOD) activity

The SOD activity was assayed using the method of Misra and Fridovich (1972).

Determination of catalase (CAT) specific activity

The CAT specific activity was assayed according to the method of Aebi (1984).

Determination of glutathione S-transferase (GST) specific activity

The GST specific activity was assayed using the method of Habig et al. (1974).

Statistics

Data are presented as mean values ± S.E.M. Student's t-test (unpaired) was applied for single comparisons. One-way ANOVA followed by Student–Newman–Keuls post-hoc test was applied for multiple comparisons. Differences were assumed to be significant for $p < 0.05$.

RESULTS AND DISCUSSION

Haematological Study

Table 1 Haematological activities recorded during exposure to oil-contaminated meal.

GROUPS	White Blood Cell (cells/µL ± sem)	Red Blood Cell (10^6/µL ± sem)	Hemoglobin (g/dl ± sem)	Hematocrit (%± sem)
CONTROL	6.64±2.38	3.58±0.27	7.57±0.19	25.27±0.79
Group 2	3.66±2.37*	2.89±1.45	5.30±2.62*	17.93±9.01*
Group 3	8.14±1.99*	4.28±1.14	8.65±1.95	27.55±5.35*
Group 4	7.20±4.02	5.36±0.34*	9.83±0.27	31.53±0.38*
Group 5	0.60±0.17*	0.39±0.20*	0.75±0.05*	2.65±0.55*
Group 6	5.13±2.43	4.09±0.78	7.60±1.70	22.80±5.20*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 (distilled water + isotonic 0.9% NaCl), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil.

Table 2 Haematological activities recorded during exposure to oil-contaminated meal.

GROUPS	Mean corpuscular volume (fl ± sem)	Mean corpuscular hemoglobin (pg ± sem)	Mean corpuscular hemoglobin conc. (g/dl ± sem)	RDWcv (µm ± sem
CONTROL	69.40±5.32	20.80±1.68	29.83±0.69	0.17±0.003
Group 2	41.67±20.83*	12.20±6.10*	19.53±9.77*	0.11±0.05
Group 3	65.75±5.05*	20.45±0.85	31.15±1.05	0.15±0.005
Group 4	59.33±3.34*	18.43±0.75*	31.13±0.47	0.15±0.003
Group 5	66.90±10.00*	19.20±0.30	29.30±3.90	0.08±0.06*
Group 6	55.30±2.10*	18.45±0.65*	33.40±0.10	0.16±0.01

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 (distilled water + isotonic 0.9% NaCl), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil.

Table 3 Haematological activities recorded during exposure to oil-contaminated meal.

Groups	RDWsd (µm ± sem	Platelets (cells/µL ± sem)	MPV (fl ± sem)	PDW (µm ± sem)	PCT (µm ± sem)
CONTROL	51.23±3.37	103.00±16.06	7.03±0.32	15.87±0.38	0.07± 0.01
Group 2	30.33±15.26*	71.00±40.15*	4.63±2.32	10.13±5.07	0.05±0.03
Group 3	41.10±0.60	435.50±260.50*	6.65±0.25	15.85±0.45	0.29±0.15*
Group 4	37.23±2.28*	253.67±118.69	6.33±0.24	15.40±0.58	0.16±0.07*
Group 5	54.50±20.20*	55.00±30.00*	6.70±0.20	15.25±0.05	0.04±0.20
Group 6	35.90±0.60*	35.50±1.50*	7.05±0.55	15.35±0.05	0.03±0.005

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 (distilled water + isotonic 0.9% NaCl), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 4 Haematological activities recorded during withdrawal from oil-contaminated meal.

Groups	White Blood Cell (cells/µL ± sem)	Red Blood Cell (10^6/µL ± sem)	Hemoglobin (g/dl ± sem)	Hematocrit (%± sem)
MI(control)	18.59±0.21	5.16± 2.57	9.90±4.40	31.75±17.65
Group 2	1.84±0.10*	5.17±0.55	8.80±0.44	26.80±0.48*
Group 3	1.90±1.77*	2.23±2.20*	3.80±3.80*	11.35±11.35*
Group 4	3.40±0.18*	3.55±0.26*	7.40±0.32*	19.80±0.57*
Group 5	16.38±0.42	5.28±0.17	9.35±0.35	28.80±2.30*
Group 6	19.38±0.67	5.96±0.31	10.90±0.38	33.20±0.37

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 5 Haematological activities recorded during withdrawal from oil-contaminated meal.

Groups	Mean corpuscular volume (fl ± sem)	Mean corpuscular hemoglobin (pg ± sem)	Mean corpuscular hemoglobin conc. (g/dl ± sem)	RDWcv (µm ± sem
MI(control)	59.00±4.90	19.58±1.35	34.05±5.05	0.16±0.01
Group 2	51.80±0.36	17.0±0.44	32.90±0.40	0.14±0.01
Group 3	25.55±25.51*	8.55±8.55*	16.75±16.15*	0.08±0.08
Group 4	55.90±0.67	20.80±0.34	37.30±0.69	0.14±0.01
Group 5	53.15±2.65	17.70±0.10	33.40±1.56	0.15±0.02
Group 6	55.70±0.10	18.30±0.12	32.80±0.13	0.20±0.23

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1, **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 6 Haematological activities recorded during withdrawal from oil-contaminated meal.

Groups	RDWsd (µm ± sem	Platelets (cells/µL ± sem)	MPV (fl ± sem)	PDW (µm ± sem)	PCT (µm ± sem)
MI(control)	0.16±0.01*	951.50±172.50	6.95±0.35*	15.50±0.12*	0.66±0.99
Group 2	0.14±0.01*	35.00±0	7.50±0	15.00±0.01*	0.03±0.21*
Group 3	0.08±0.08*	12.80±11.00*	3.55±3.55	7.65±7.65*	0.01±0.01*
Group 4	0.14±0.12*	297.00±0.12*	6.70±0	15.30±0	0.20±0
Group 5	0.15±0.02	514.50±35.50*	6.30±0.10	15.30±0.10	0.33±0.02*
Group 6	0.20±0.01	697.00±0	7.1±0	15.20±0	0.49±0

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 7 Some selected electrolytes' activities during Crude oil exposure in graded feed contamination.

Groups	Bilirubin (mg/dl ± sem)	Na ions (mmol/L ± sem)	K ions (mmol/L ±sem)	Cl (mmol/L ±sem)	HCO3 (mmol/L ± sem)
CONTROL	0.44±0.02	146.33±2.78	5.1±1.11	103±4.44	22±1.10
Group 2	0.72±0.02	143.33±3.92	4.9±1.11	99.67±2.47*	21.33±1.10
Group 3	0.7±0.02	152.5±2.47	6.3±0.02	105.5±2.47	18.5±1.00
Group 4	0.7±0.02	152.67±4.84	5.9±0.02	106±5.92	19.67±0.80
Group 5	0.9±0.02	149±2.25	5.1±0.03	103.5±4.44	22±0.80
Group 6	0.88±0.02	151±2.68	5.75±0.00	104±4.40	19.5±0.60

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 (distilled water + isotonic 0.9% NaCl), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil.

Table 8 Some selected electrolytes' activities during Crude oil exposure in graded feed contamination.

Groups	Bilirubin (mg/dl ±sem)	Na ions (mmol/L±sem)	K ions (mmol/L±sem)	Cl (mmol/L±sem)	Hco3 (mmol/L±sem)
MI(control)	1.13±0.01	151±1.12	5.4±0.01	99±2.33	23±0.80
Group 2	0.52±0.02	122±1.34*	5.9±0.01	110±1.34	16±0.20*
Group 3	0.12±0.02*	159±0.80	5.8±0.01	104.5±2.33	19.5±0.50
Group 4	0.17±0.01*	158.5±1.11	5.8±0.02	104.5±2.44	21±0.55
Group 5	1.06±0.00	154±1.15	5.5±0.02	102.5±2.07	20±0.02
Group 6	0.42±0.00*	154±1.12	6.05±0.00*	104±2.22	21±0.02

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 9 Biochemical activities of liver enzymes, urea, and creatinine after exposure to crude oil meal.

Groups	Urea(mg/dl ± sem)	Creatinine (mg/dl±sem)	AST (U/L ± sem)	ALT(U/L ± sem)	ALP(U/L ± sem)	Protein (g/l ± sem)
CONTROL	25.15±1.78	8.66±0.02	63±0.02	17.33±1.23	12.27±2.35	3.22 ± 1.12
Group 2	33.12±1.78*	4.74±0.02*	90.33±0.02	31.67±11.21*	23.15±6.25	4.51 ± 2.24
Group 3	33.1±3.45*	11.57±0.01*	90.5± 1.23	65.23± 10.13	29.9 ±7.82	4.93 ± 1.11
Group 4	48.4±0.10*	17.58±0.00*	85.33±1.00	19.12 ± 5.23	30.67±5.45	4.65 ±1.15*
Group 5	50.06±4.44*	24.36±0.00*	85.5±3.12	12.51 ± 3.45	41.13±8.26	4.95 ±1.32*
Group 6	56.71±2.01*	12.59±0.20*	85±2.12	47.53 ± 5.48	64.12±3.33	5.53 ±1.18*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Table 10 Biochemical activities of liver enzymes, urea, and creatinine after withdrawal of crude oil meal.

GROUPS	Urea(mg/dl ± sem)	Creatinine (mg/dl ±sem)	AST(U/L ± sem)	ALT(U/L ± sem)	ALP(U/L ± sem)	Protein(g/l ± sem)
MI(control)	21.89 ± 7.34	1.03 ± 0.11	60.5 ±2.12	7.44 ±2.11	5.28±1.11	1.24 ± 0.22
Group 2	11.65±3.25*	1.76 ± 0.11	80.0±12.02*	12.21±7.80*	6.28±1.09	3.18 ± 1.12*
Group 3	11.12±4.12*	2.44±1.02*	90.0±12.06*	17.5 ± 5.55	5.98±9.05	3.24 ± 0.77*
Group 4	18.18±3.56*	1.08 ±0.01	90.5 ±9.07*	32.5 ± 7.43*	6.44±1.22	3.05 ± 1.10*
Group 5	15.89±3.90*	1.95 ±0.22	90.5 ±8.22*	42.5 ± 5.89*	13.8±4.73*	3.83 ± 0.23*
Group 6	20.83 ±5.18	5.76±1.6*	87.5 ±6.67*	51.11±10.2*	15.98±3.54*	3.42 ± 1.13*

Values are presented in mean ± sem, n= 5. * means values are statistically significant when compared to the control.

Key; **group 1** control 1 **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 11 Oxidative stress markers' activities in the exposed and control during exposure to crude oil meal.

GROUPS	Malonaldehyde (µg/ml ±sem)	Sodium dismutase (u/ml ±sem)	Catalase (u/g ±sem)	Glutathione reductase (µg/ml ±sem)	Glutathione peroxidase (µg/ml±sem)
CONTROL	210.23±74.83	2.72±0.95	0.02±0.009	0.11±0.10	0.06±0.02
Group 2	66.36±16.09*	1.65±0.03	0.03±0.02	0.11±0.005	0.05±0.003
Group 3	15.98±1.65*	2.18±0.51	0.31±0.002	0.11±0.005	0.04±0.006
Group 4	36.90±0.32*	2.11±0.03	0.10±0.00	0.13±0.009	0.03±0.017
Group 5	63.25±26.24*	3.18±1.23	0.10±0.005	0.12±0.005	0.03±0.007
Group 6	12.77±0.15*	2.18±0.16	0.18±0.04	0.11±0.015	0.05±0.026

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control

Key; **group 1** control 1 (distilled water + isotonic 0.9% NaCl), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Table 12 Oxidative stress markers' activities during exposure to crude oil meal

GROUPS	MDA (µg/ml±sem)	SOD (u/ml±sem)	Catalase (u/g±sem)	GSHred (µg/ml±sem)	GSHperox (µg/ml±sem)
MI(control)	1.80±0.00	1.77±0.00	0.02±0.00	0.11±0.00	0.1±0.00
Group 2	3.19±0.00*	21.43±0.00*	0.09±0.00	0.10±0.00	0.09±0.00
Group 3	1.80±0.00	16.79±0.00*	0.03±0.00	0.11±0.00	0.04±0.00
Group 4	2.04±0.00	15.91±0.00*	0.05±0.00	0.12±0.00	0.05±0.00
Group 5	1.90±0.00	11.25±0.00*	0.08±0.00	0.01±0.00	0.05±0.00
Group 6	1.54±0.00	22.27±0.00*	0.09±0.00	0.12±0.00	0.03±0.00

Values are presented in mean ± sem. n= 5. P ≤ 0.05 *means values are statistically significant when compared to the control

Key; **group 1** control 1 **MI** (epinephrine-induced myocardiac infarct group), **Group 2** (BLCO, 3.88g/kg only), **Group 3** (BLCO, 7.75g/kg only), **Group 4** (BLCO, 15,51g/kg only), **Group 5** (BLCO, 32.01g/kg only), **Group 6** (BLCO, 62.02g/kg only). **BLCO** = Bony light Crude Oil

Crude oil contaminated meal at graded doses had apparently delirious effect on the blood cells. The present study showed that crude oil, BLCO may have altered the structure of the membrane of endoplasmic reticulum (ER) and mitochondria which stored calcium considering its deleterious

effect on the membrane. The analysis of results reveals that during the period of exposure of the animals to crude oil meal at different concentrations the level of WBC slightly but insignificantly varied across the test groups and the control with an exception of group 5. This pattern of variation was the same for the concentrations of other parameters.

The red blood cell concentration also followed similarly in pattern. Results reveals that during the period of exposure to crude oil meal, the level of Bilirubin, Sodium, Potassium, Bicarbonate and Chloride ions remained relatively unchanged across board. The electrolyte profile was seen not to be aggravated or significantly reduced which could account chemical equilibrium across the cell membrane. Crude oil has been previously established to exert toxic effect on membrane architecture (Khan et al., 2001).

There was a slow progression towards the control values in the levels of electrolytes in the groups fed with crude oil meal. There was a remarkable reversal from the low levels of the electrolytes in the exposed phase when compared with that of withdrawal phase.

Liver enzymes were estimated and the data analysis of results reveals that during the period of exposure of crude oil meal, unlike the level of Urea and creatinine that increased significantly in the test groups compared to the control, the pattern of variation presented that of transient increase for AST, ALP and ALT. The Protein level remained relatively unchanged across board. Assessment of plasma and liver enzyme activities can be considered as diagnostic tool to determine the physiological status of cells or tissue (Whitby et al., 1984)).

The significant increase in plasma enzyme activity indicated that crude oil stimulated aspartate aminotransferase which are Mitochondria enzymes. The increase in the plasma could be due to toxic injury caused by crude oil which stimulated tissue repair through protein turnover and increased respiration. Liver enzymes levels are usually raised in acute hepatoxicity, but tend to decrease with prolonged intoxication due to damage to the liver (Bronk et al., 1999).

Enzyme activities in tissues are often used as marker to ascertain early toxic effects of administered foreign compounds to experimental animals. Results obtained showed a significant increase ($p<0.05$) in the levels of urea, creatinine and electrolytes (Na^+, K^+ and CO_3^{2-}) in the serum of rats administered the crude oil meal compared to control. This is similar to the study of a Haines (2001) which reported that exposure of various stressor usually elicits changes in liver enzymes with the lowest concentration having the highest effect. Although liver pathology, hepatocellular lipid vacuolization was suggested by Jacobs, (2005) to be non- specific liver lesions induced by exposure to a variety of hydrocarbon at toxic levels.

Oxidative stress markers were estimated and the data analysis of results reveals that during the period of exposure to crude oil meal, the level of MDA, SOD, Catalase and Glutathione varied slightly and insignificantly across the test groups compared to the control. Glutathione oxidase and peroxidase levels remained relatively unchanged across board. There are indications that constant exposure of man and other animals that share common features with man to crude oil could lead to oxidative stress (Sies, 1997).

From tables 11 and 12, Oxidative stress markers were estimated and the extrapolates showed that during the period of withdrawal from crude oil meal, the level of SOD, Catalase and Glutathione increased significantly across the test groups compared to the control while MDA, Glutathione oxidase and peroxidase levels remained relatively unchanged across board.

Observations from this study revealed that exposure to petroleum hydrocarbon led to oxidative damage of the heart evident by a rise in MDA, and reduction in glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities. This agrees with a previous study [Ridker *et al*, 2001] that reported the pro-oxidant effect of hydrocarbons.

After the withdrawal of the crude meal, there was a reduction in the activities of MDA and a significant rise in the levels of catalase SOD, and glutathione enzymes which could be suggestive of a marked amelioration of lipid peroxidation following the withdrawal of crude oil meal Oxidative stress, characterised by the presence of reactive oxygen species (ROS) in excess of the available antioxidant-buffering capacity [Acworth and Bailey, 1997], can damage molecular targets—proteins, lipids and DNA, thus altering the structure and function of the cell, tissue, organ, or system [Breimer, 1990].

CONCLUSION

The overall results demonstrated the debilitating consequences our polluted environments can impose on our collective existence and survival. Crude oil is not readily biodegradable and the effects of exposure to this toxin will be felt from generation to generation. Crude oil contaminated meal at graded doses had apparently delirious effect on the blood cells. From tables 1-6, it appeared initially as if there would not be significant interruption in these cells' intracellular chemical activities but on the long run, there appeared to be 'weakening' on their membrane integrity.The haemoglobin concentrations alongside the red and white blood types had a significant loss in quantity in groups as the crude oil concentration in the meals increased. This observation could be triggered by the myriads of challenges termed oxidative stress these cells were exposed to in terms of cellular toxicity as previously observed by Clarkson, (1995).

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