RESEARCH ARTICLE Am. J. PharmTech Res. 2018; 8(5) ISSN: 2249-3387

AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Effect of Bony Light Crude Oil (BLCO) Contaminated Feed on Cardiovascular Integrity and Risk Factors in Wistar Rats

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ABSTRACT

The effect of crude oil contaminated feed on cardiovascular integrity and risk factors in wistar rats 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 Treatments in various groups were administered for 8 weeks (exposure period) and were later withdrawn for 2 weeks (withdrawal period). The blood pressures (Systolic, diastolic blood pressure and heart rate) were recorded in both phases, 5 ml of blood was taken from all groups via cardiac puncture in both phases for analysis of lipid profiles. Cardiovascular Risk Indices (Castelli Risk index I & II, Atherogenic index of plasma, and atherogenic coefficient) 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 $P \leq 0.05$. Blood pressure estimates and lipid parameters all presented marked increase during the exposure phase of six weeks. Similarly, cardiovascular risk indices were aggravated significantly ($P \leq 0.05$) during the same period. 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 lipid peroxidation, electrolyte imbalance, cellular disruptions, and can be highly detrimental and delirious to cells while withdrawal from the contaminated meal was observed to reverse the entire scenarios. In conclusion, crude oil contaminated feed on cardiovascular integrity and risk factors could be a major pre-disposing scenario for biologic derangement and down-regulation of cellular bio-functions in living organisms at estuaries and among riverine dwellers.

Keywords: Crude oil, Castelli Risk index I & II, Atherogenic index of plasma, and Atherogenic coefficient

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Received 20 August 2018, Accepted 29 August 2018

Please cite this article as: Olorunfemi OJ *et al.*, Effect of Bony Light Crude Oil (BLCO) Contaminated Feed on Cardiovascular Integrity and Risk Factors in Wistar Rats. American Journal of PharmTech Research 2018.

INTRODUCTION

Myocardial infarct as characterized by gross cardiac lipotoxicity is regarded as sum of various cell-damaging processes that includes disruption of organelle membranes and activation of the apoptotic machinery, cellular stress and oxidative stress have been identified as crucial components of the lipotoxic response (Brookheart *et al*., 2009) Different enzymatic sources of oxidative stress including NADPH oxidase (Murdoch *et al*, 2006), uncoupled nitric oxide synthase (NOS; (Takimoto *et al*, 2005), and xanthine oxidase (Engberding *et al*., 2004), have been implicated in the development of cardiac dysfunction. Recent evidence points to an essential role of NADPH oxidases as mediators of lipid-driven oxidative stress.

Toxic intermediate products from crude oil may further worsen cardiac function and metabolism with development of progressive myocardial atrophy and protein breakdown (Zhou et al., 2000). Although the hemodynamic improvement through unloading of the left ventricle by left ventricular assist device implantation partially corrects pathways of protein synthesis and degradation (Razeghi *et al.*, 2005), no data are known regarding the impact of hemodynamic correction on lipid accumulation and markers of lipotoxicity. Researchers also attempted 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)

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 renewed 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)

Blood Pressure Measurement

The systolic and diastolic blood pressure and heart rate were measured by Ugo Basile non-invasive blood pressure recorder apparatus before and after treatment and in both phases too.

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 append or zf tubes, stored at -20OC until used for the analysis of Cholesterol, Triglycerides, LDL, HDL.

Estimation of cardiovascular risk indices

Atherogenic Index of Plasma AIP, was calculated using the relation, log TG/HDL Castell Risk Index I, using Tc/HDL, Castelli Risk Index II, using LDL/HDL and Atherogenic Coefficient, using Tc–HDL/HDLc.

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

BLOOD PRESSURE STUDY

Table 1 Cardiovascular Parameters as presented by various groups after exposure to crude oil meal.

GROUPS	SYSTOLIC BP (mmHg±sem)	DIASTOLIC BP (mmHg±sem)	HEART RATE (bpm±sem)
CONTROL	122.80±23.33	93.20±8.17	397.80±23.87
Group 2	208.80±24.72*	75.80±2.96	726.60±9.88*
Group 3	145.00±16.27*	76.75±3.28*	404.50±123.52
Group 4	134.40±25.88*	93.00±20.73	378.40±108.63

Group 5	151.25±7.49*	97.50±22.6	367.75±13.91
Group 6	171.50±17.53*	75.00±4.13*	465.75±94.92*

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 Cardiovascular Parameters as presented by various groups after withdrawal of crude oil meal.

GROUPS	Systolic BP (mmHg ± sem)	DIASTOLIC BP (mm Hg ± sem)	HEART RATE (bpm ± sem)
Control(MI)	153.00±45.00	120.00±41.00	255.50±115.50
Group 2	200.00±0.00*	80.00±0.00*	333.00±0.00*
Group 3	183.00±14.00*	121.0±40.0*	522.00±78.00*
Group 4	149.50±13.5	82.00±0.00*	727.50±22.50*
Group 5	116.50±25.50*	79.50±0.50*	638.00±67.00*
Group 6	177.50±36.50*	80.50±0.50*	191.00±133.00*

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 3 Lipid profile of the test and control groups during exposure to crude oil meal

GROUPS	Cholesterol (mg/dl±sem)	Triglyceride (mg/dl sem)	HDL (mg/dl±sem)	LDL (mg/dl ±sem)
CONTROL	3.87±1.94	9.12±1.01	5.25±0.003	1.90±0.81
Group 2	7.60±0.23*	11.46±1.67	3.21±1.01	4.73±2.59*
Group 3	8.20±0.91*	11.23±2.34	3.65±0.32	5.95±2.84*
Group 4	7.39±0.44*	10.10±1.77	3.88±1.45*	5.74±0.73*
Group 5	6.98±0.91*	9.88±2.49	3.02±3.02*	5.45±5.69*
Group 6	7.97±0.08*	11.53±1.28*	2.25±0.02*	7.69±1.25*

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 Lipid profile of the test and control groups during exposure to crude oil meal

GROUPS	Cholesterol (mg/dl±sem)	Triglyceride (mg/dl±sem)	HDL (mg/dl±sem)	LDL (mg/dl±sem)
MI(control)	14.91±0.61	8.47±0.60	3.44±0.52	7.87±0.25
Group 2	15.22±0.00	8.42±0.00	3.31±0.00	9.71±0.00
Group 3	12.89±0.53	6.96±0.32	3.72±0.44	8.35±0.96
Group 4	14.10±0.53	6.69±0.45	3.52±0.50	9.92±0.05
Group 5	15.90±0.83	6.45±0.07	3.82±0.74	9.73±0.02
Group 6	17.89±0.98	6.32±0.19	3.72±0.50	9.00±0.05

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 5 Cardiovascular Risk Indices as presented by various groups after exposure to crude oil meal

GROUPS	Atherogenic Index Of Plasma (AIP) {log TG/HDLc}	Castelli's Risk Index I (CR-I) {TC/HDLc}	Castelli's Risk Index II (CR-II) {LDLc/HDLc}	Atherogenic Coefficient (AC) {TC-HDLc/HDLc}
CONTROL	0.24±0.01	1.74±0.02	0.36±0.00	0.74±0.03
Group 2	0.55±0.00	3.57±0.02*	1.47±0.02	2.57±0.02
Group 3	0.49±0.02	3.08±0.01*	1.63±0.02	2.08±0.02
Group 4	0.42±0.00	2.60±0.02	1.48±0.01	1.60±0.01
Group 5	0.51±0.02	3.27±0.00*	1.80±0.00*	2.27±0.02*
Group 6	0.71±0.01	5.12±0.00*	3.42±0.02*	4.12±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 (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 6 Profile of Cardiovascular Risk Indices after one week withdrawal of crude oil meal

Groups	Atherogenic Index Of Plasma (AIP) {log TG/HDLc}	Castelli's Risk Index I (CR-I) {TC/HDLc}	Castelli's Risk Index II (CR-II) {LDLc/HDLc}	Atherogenic Coefficient (AC) {TC-HDLc/HDLc}
MI(control)	0.39±0.02	2.46±0.02	2.29±0.10	1.46±0.02
Group 2	0.41±0.02	2.54±0.02	2.93±0.08*	1.54±0.02
Group 3	0.27±0.00	1.87±0.00	2.24±0.07	0.87±0.00

Group 4	0.28±0.01	1.90±0.01	2.81±0.10*	0.90±0.01
Group 5	0.23±0.01	1.69±0.02*	2.54±0.10	0.69±0.02
Group 6	0.23±0.00	1.70±0.02*	2.42±0.02	0.70±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

COMPARATIVE STUDY

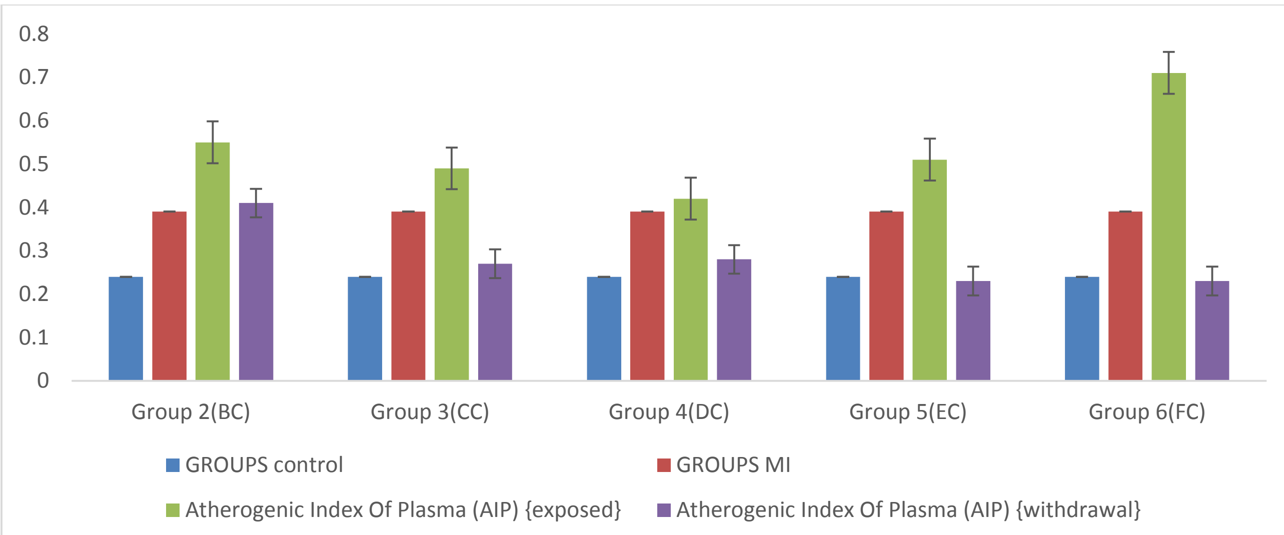

Figure 1 Comparison of Atherogenic index of plasma during exposure and withdrawal phases.

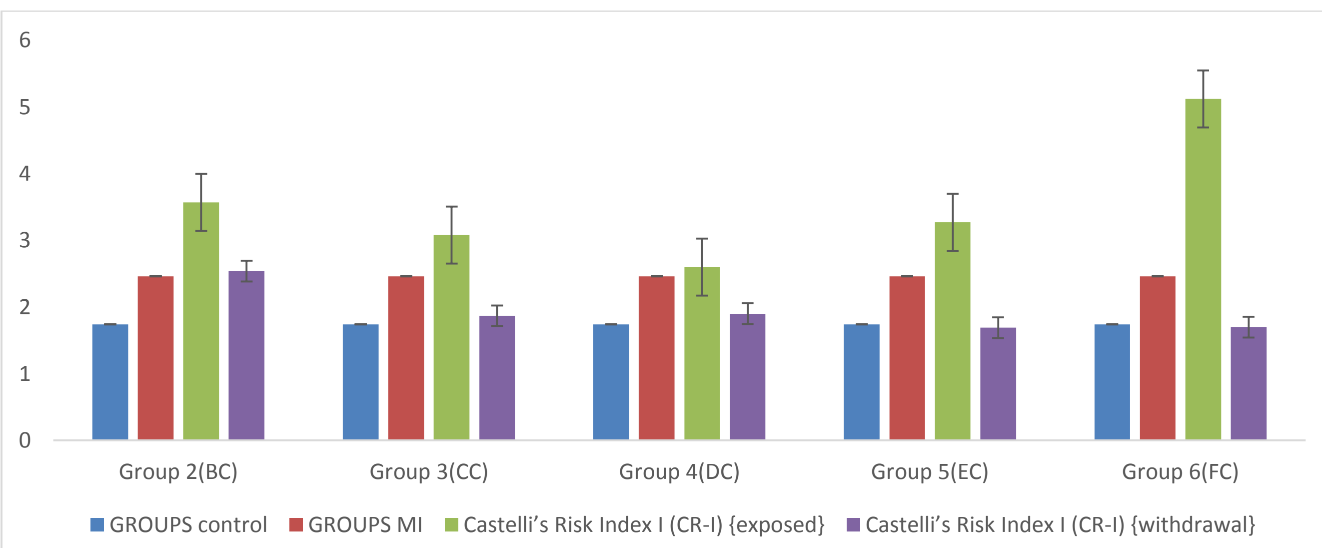

Figure 2 Comparison of castelli Risk index I during exposure and withdrawal phases.

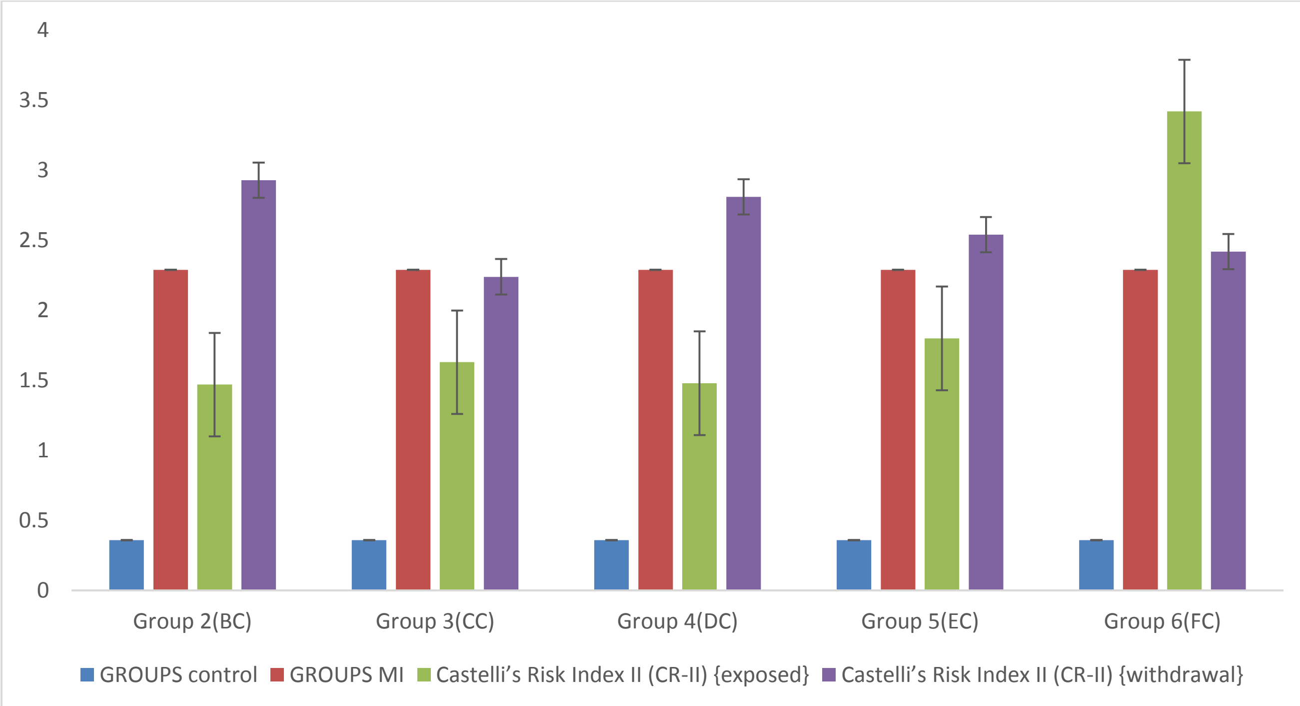

Figure 3 Comparison of castelli Risk index II during exposure and withdrawal phases.

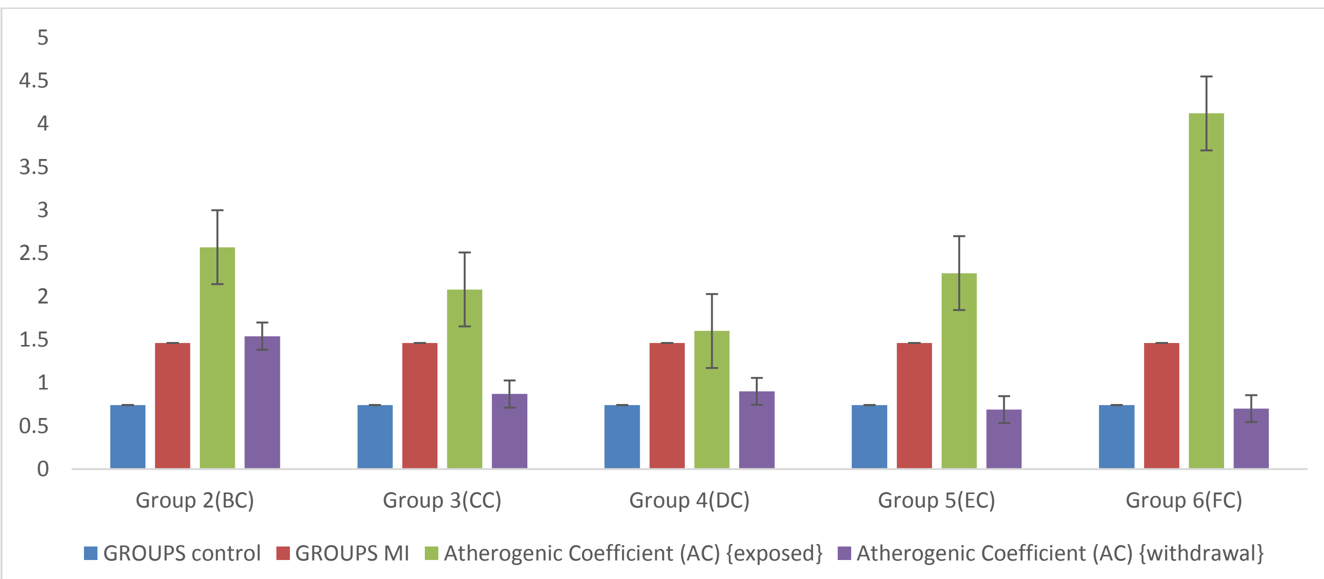

Figure 4 Comparison of Atherogenic Coefficient during exposure and withdrawal phases.

The effects of crude oil (BLCO) contamination on cardiovascular integrity and risk factors in wistar rats were investigated and the resultant observations were extrapolated, analysed, interpreted and presented in this section.

Both Systolic and diastolic blood pressures were significantly increased during the exposure phase to crude oil meal. The heart rate subsequently increased too across the exposed groups compared to the control (tables 1 and 2).

The biochemical response on exposure to crude oil meal aggravated the significant increase in cholesterol, triglyceride, and low density lipoprotein levels with a concomitant decrease in the level of high density lipoprotein. This clearly demonstrated lipid peroxidation. Cell development certain lipids are indicators of cellular events, and lipid concentration can represent physiological conditions of cells (wolfrum & Spener, 2000), Lipids are atherogenic hence could cause high blood pressure by forming plaques that narrow the blood vessels (Egbuonu and Ezeanyika, 2012).

The comparative results between the exposed phase and the withdrawal phase (figures 4.34-4.39), revealed that marked arrest in lipid peroxidation were observed which were useful to the animals to improve in their HDL level. Lipid peroxidation usually results in decreasing membrane fluidity, cell injury and may cause the formation of atherosclerotic plaques (Kris-Etherton, 1999).

Castelli Risk index I and II (CRI – I and II) are new parameters in assessing cardiovascular risk. Castelli Risk Index – I is based on the ratio of two important parameters Total cholesterol (TC) and High Density Lipoprotein cholesterol (HDLc), both of which are independent risk factors for Coronary Artery Disease (CAD). CRI-II, calculated as the ratio of Low Density Lipoprotein cholesterol (LDLc) and (HDLc) is another fraction which involves risk factors for predicting CAD. In the present study, parameters such as cholesterol, triglycerides and low density lipoprotein that predispose to myocardial infarct were significantly high compared to the control. Studies have shown an inverse relationship that exist between TG and HDLc and that the ratio of TG to HDLc is a strong predictor of infarction (Gaziano et al., 1997).

Castelli Risk index I and II (CRI – I and II) were observed to be significantly high in all groups exposed to crude oil meal as compared to the control. The marked decrease in these indices following the withdrawal of the meal led to recovery from the derangement.

The Patterns of atherogenic index of plasma and atherogenic coefficient, which were high significantly during the crude oil meal exposure were progressively reduced after withdrawal. The increment observed could have been triggered by the significant increase in cholesterol and LDL as observed across the groups. The overall results demonstrated the debilitating consequences our polluted environments can have 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.

CONCLUSION

Crude oil contamination has a direct debilitating effect on the heart both on short and long term exposure. The heart faces a mammoth of physical and biochemical stress and assault in delivering its pumping functions and this could lead to derangement. Values from Castelli Risk Index I and II proposed that the health status of the hearts of the exposed animals could be at risk of myocardial infarction, these values are significant predictors of coronary artery disease (CAD). Sooner or later, crude oil products over time when in constant contact with the human body could become contaminants that can potentially impair overall body functions such as heart functions and initiating other debilitating effects.

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