

**HAEMATOLOGICAL AND KIDNEY FUNCTION MARKERS
EVALUATION OF CRUDE OIL CONTAMINATED FEEDS
SUPPLEMENTED WITH ANTIOXIDANTS IN ALBINO WISTAR
RATS.**

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ABSTRACT

This study investigated the ameliorative effect of antioxidant on crude oil contaminated feeds in wistar rats. Forty male albino rats were randomly assigned to five experimental groups of eight rats each namely; Control which was fed with normal feeds, crude oil control (negative control) which was fed with contaminated feeds, and the treatment groups which were fed with contaminated feed and treated with; 0.5mg of garlic and 1.7mg of Vitamin C and E respectively. After 7 and 14 days, body weights were taken and rats sacrifice under anesthesia. The hormonal level, kidney function enzymes and

hematological indices were determined. It was observed that crude oil contamination significantly ($p < 0.05$) reduced the body weight of the wistar rats, and the antioxidants improved it, although not significantly ($p < 0.05$). The result also showed that crude oil significantly ($p < 0.05$) reduced the testosterone and FSH level in the rats on day 7 and 14, which the antioxidants significantly improved ($p < 0.05$). Anemia was also observed in rats fed with crude oil contaminated feeds which were also revived by antioxidants although not significant ($p < 0.05$) improvement. The kidney function enzymes were also observed and there was a significant ($p < 0.05$) increase in the blood urea nitrogen level and the creatinine level which further significantly increased ($p < 0.05$) when treated with vitamins C and E. In conclusion, Antioxidants such as Vitamin C, E and Garlic improved body weight,

reproductive hormones, hematological and Kidney function enzyme levels in male albino wistar rats fed with crude oil contaminated feeds.

KEYWORDS: *Body Weight Indices, Hematological Indices, Hormonal Assay, Kidney Function Markers.*

INTRODUCTION

Crude oil exploration is the major income of the Nigerian economy and constitutes about 90% foreign exchange earning of the nation. Crude oil, a source of returns to many other oil producing countries, could become a threat to life when it contaminates the environment and studies have shown that various environmental pollutants, particularly those associated with crude oil cause many biochemical and toxic effects in both terrestrial and marine animals. There are different means of crude oil pollution ranging from drilling, exploration exploitation and transport. Thus crude oil or liquid petroleum hydrocarbon find its way into the aquatic environment such as oceans and coastal waters as well as on and can have devastating effects on animals, plants and the environments associated with these regions where the spillage occurs (Agbogidi *et al.*, 2005). The devastating effect of crude oil is linked to its components. Crude oil is basically made of hydrocarbons with metals and other organic compound with includes polycyclic aromatic hydrocarbons (PAH) as the major contaminants. PAHs are known to be mutagenic and carcinogenic to many animals. At prolonged exposure, it is likely the animals start having problems adapting to the new conditions and hence Vitamin C, Vitamin E and Garlic's anti-oxidant cycle could be ignited (Bostrom *et al.*, 2002).

Ascorbic acid or Vitamin C has many functions ranging from Electron transport to Enzyme cofactor and anti-oxidant properties. As an anti-oxidant, it reacts with superoxide, single oxygen, ozone and hydrogen peroxide, withdrawing lone electron on them. It thus participates in removal of these reactive forms of oxygen which are generated during aerobic metabolism and during exposure to some pollutants and herbicides. Also, the ascorbic acid can participate in the removal of induced oxidative stress which are generated by reactive oxygen species (ROS). ROS can speedily attack and harm bio-molecules including proteins, lipids and nucleic acids. The likelihood of ascorbic acid to remove oxidative stress can then be tested using an economical viable model plant (Acworth and Bailey, 1997).

Vitamin E refers to a group of compounds that include both tocopherols and tocotrienols.. Vitamin E's major function appears to be as a non-specific chain-breaking, fat soluble

antioxidant that prevents the propagation of free-radical reactions (Brigelius-Flohé and Traber, 1999). As an antioxidant, vitamin E acts as a peroxy radical scavenger, disabling the production of damaging free radicals in tissues, by reacting with them to form a tocopheryl radical, which will then be reduced by a hydrogen donor (such as vitamin C) and thus return to its reduced state (Traber and Stevens, 2011). As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage. Vitamin E has also found use as a commercial antioxidant in ultrahigh molecular weight polyethylene used in hip and knee implants by resisting oxidation (Bracco, 2011). Garlic's current principal medicinal uses are to prevent and treat cardiovascular disease by lowering blood pressure and cholesterol, as an antimicrobial, and as a preventive agent for cancer. Garlic products are used as sources of medicine in many ways in human beings in their day to day life. Garlic extract have antimicrobial activity against many genera of microorganisms. It contains a high concentration of sulfur compounds which are responsible for its medicinal effects. The chemical constituents of garlic have also been investigated for treatment of cardiovascular disease, cancer, diabetes, blood pressure, atherosclerosis and hyperlipidemia and highly commended in many journals (Gebreselema and Mebrahtu, 2013).

MATERIALS AND METHODS

Experimental animals: Forty male albino rats between 110-130g were purchased from Ladoke Akintola University of Technology, Ogbomosho, Nigeria and used for the study. The rats were randomly assigned on the basis of their body weight into five (5) study groups of eight (8) rats each. They were fed with feeds (control) and feed mixed with crude oil and distilled water and were treated with 0.5ml garlic solution, 0.5ml ascorbic acid solution and 0.5ml vitamin E solution. They were kept in secure wooden cages of 10 rats per cage placed in a well-ventilated animal room of Joseph Ayo Babalola University at normal temperature of 30-35°C. The cages were cleaned daily and the rats were treated according to the international guidelines for the care and use of laboratory animals (NIH, 2008). The animals were allowed for two weeks of acclimatization and their weights were measured before treated commenced.

Crude oil Sample: The test sample, crude oil, was obtained from the Port Hacourt terminal, well 4 of SHELL oil. A class A oil, light and volatile.

Source of the drugs: Vitamin C, E and garlic Capsule were purchased from SETZ Pharmaceutical Limited, Ikorodu, Lagos and used for the study.

Chemicals: Chloroform and other chemicals of analytical grade were obtained from Fam-lab Nigeria Limited.

Formulation of contaminated diet: A 10% contamination was achieved by measuring 5ml of crude oil and thoroughly mixed with 45g of animal feeds. The feeds for the control group contained no crude oil (i.e. 0% group).

Experimental design: The grouping and feeding of crude oil contaminated feeds and antioxidant treated group is shown as follows.

Group A: Designated as positive control group fed with normal feeds and water *ad-libitum*.

Group B: Designated as negative control group fed with crude oil contaminated feeds and water **Group C:** Designated as crude oil contaminated group fed with 0.5mg garlic

Group D: Designated as vitamin C group fed with a portion of crude oil contaminated feed and given 1.7mg vitamin C treatment and water *ad-libitum*.

Group E: Designated as vitamin E group fed with a portion of crude oil contaminated feed and given 1.7mg vitamin E treatment and water *ad-libitum*.

Sacrifice of the animals: At the end of the experimental period, rats in each study group were fasted overnight and sacrificed under anesthesia by cardiac puncture.

Blood collection: After the rats have been sacrificed, 3-4ml of blood was collected from each rat and placed in sterile plain bottles for kidney enzymes analysis and EDTA bottles for hematological indices.

Determination Haematological Indices

Determination of blood haemoglobin: Blood haemoglobin determination was carried out by the cyanomethaemoglobin method of Crosby *et al.*, 1954 as modified by Pla and Fritz, 1971.

Determination of haematocrit- packed cell volume: The method permits the determination of the volume occupied by the red blood cells (RBC's) in the blood. A capillary tube was filled with whole blood and spun in a centrifuge to pack the red blood cells. The haematocrit value which is the ratio of the height of cells over the total height of fluid in the tube was read and converted from a micro-haematocrit reading device.

Determination of total white blood cell count: 0.02ml of the blood was added to 0.38ml of the solution in the test tube. It was mixed and left at room temperature, then the counting

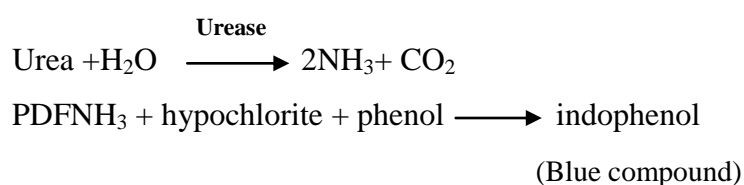
chamber was charged with the aid of the capillary tube the cell contained in four other squares of the chamber was counted under the microscope using a X10 objectives.

Determination of deferential white cell count: 0.15g of Leishman's powder was weighed into 100ml of methanol. It was mixed thoroughly and filtered into brown bottle corked and stored in a dry place. A drop of blood was placed on a slide with the aid of cover slip, a blood smear was made at an angle of 45° and allowed to air dry. The film was flooded with Leishman stain solution for 3-5mins, it was diluted with buffer.

The film was flooded with Leishman stain solution for 3-5mins, it was diluted with buffer solution and left for 10-15mins and it was washed gently under running tap water. It was then allowed to dry and examined under microscope using X100 (oil immersion) objectives.

Determination of kidney function enzymes level

Determination of blood urea nitrogen: Urease Berthelot method was used where urea in serum was hydrolyzed to ammonia in the presence of urease. The ammonia was then measured photometrically by Berthelot's reaction



Determination of creatinine: Colorimetric method. Jaffe was used where Creatinine in alkaline solution reacted with picric acid to form a coloured complex. The amount of the complex formed is directly proportional to the creatinine concentration.

Hormonal assay

Testosterone estimation: The testosterone EIA was based on the principle of competitive binding between testosterone in the test specimen and testosterone-HRP conjugate for a constant amount of rat anti-testosterone.

Follicle stimulating hormone estimation: The FSH quantitative test kit was used based on the principle of a solid phase enzyme-linked immunosorbent assay.

Body weight indices: The body weights of experimental rats were measured and recorded at day 0, day 7 and day 14 respectively.

Physicochemical analysis of crude oil

pH estimation: The pH of the sediment samples in the crude oil was determined by the method describes by Hendershot *et al* (1993). From the sub sample, 10g sample was weighed into a 100ml beaker and 20ml distilled water added. The mixture was properly stirred and allowed to stand for 30 minutes. The pH meter was first of all calibrated with buffer solutions at pH 4 and 9, and this was followed by dipping the electrode into the sample solution and the pH read on a Jenway pH meter.

Total solids determination: This was determined gravimetrically by evaporating a known volume of the crude oil sample to dryness and further drying to constant weight in the oven at 105°C. A clean evaporating dish of over 100ml capacity was first dried at 105°C in an oven to constant weight. This was weighed after cooling in a desiccator. 100ml of the thoroughly mixed sample were measured into the dish and evaporated to dryness on a steam bath. The residue in the dish was then oven dried at 105°C to a constant weight and the total solid in mg/L was then calculated from the difference in weight of the dish and dish plus residue.

Total suspended solids determination: Suspended solids or non-filterable solids of the crude oil samples were determined gravimetrically after filtering a known volume of the sample through a glass fibre or glass filter paper. The glass fibre was first washed with distilled water, dried at 105°C in an electric oven, cooled in a desiccator and weighed. 100ml of the crude oil sample after thorough mixing were then measured and filtered through the glass fibre, and the filter with the residue dried to a constant weight at 105°C in the oven. The difference between the weight of the glass fibre before and after filtration gave the total suspended solid.

Total dissolved solid Determination: Total dissolved solids was obtained by difference between total solids and total suspended solids ($TDS = TS - TSS$).

Turbidity Determination: Turbidity was determined by the Nephelometric method using a potable HACH meter (DR/890) Data logging colorimeter. The instrument was first calibrated with a standard turbidity suspension supplied with the instrument after setting to read turbidity. The sample was then thoroughly shaken so as to disperse the solid contents and some quantity was poured into the turbidimeter tube and the turbidity read from the instrument. Distilled water was used to zero the instrument in the measurement.

Bicarbonate determination: Alkalinity was first determined titrimetrically in the crude oil sample using the indicator method. Carbonate and hydroxide were determined by titrating 50ml of the sample with standard 0.01M H₂SO₄ after the addition of 3 drops of phenolphthalein indicator. This gave the phenolphthalein alkalinity. Methyl orange or Bicarbonate alkalinity followed immediately after the phenolphthalein alkalinity determination by the addition of 3 drops of methyl orange into the sample and continued the titration with the standard H₂SO₄ from yellow to orange-red colour end-point.

Sulphate determination: Sulphate in crude oil sample was determined by turbidimetric method.

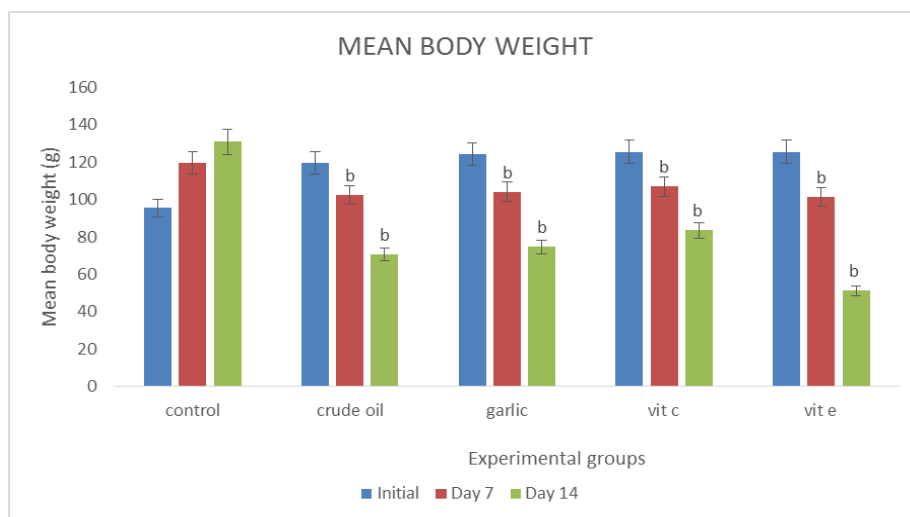
Chemical oxygen demand determination: The COD in the crude oil sample was determined titrimetrically, the process being achieved by using a strong oxidizing agent (potassium dichromate) under acidic condition.

RESULTS

Table 1.0: Physiocochemical analysis of crude oil.

S/N	PARAMETER	SAMPLE RESULTS
1.	pH	9.250
2.	Temperature (°C)	29.900
3.	Dissolved Oxygen (Mg/L)	1241.460
4.	Cod (Mg/L)	169200.000
5.	E. Conductivity (µscm)	1950.000
6.	Turbidity (Ntu)	220.000
7.	Total Solids (Mg/L)	1104.000
8.	T. Dissolved Solids (Mg/L)	975.000
9.	T. Suspended Solids (Mg/L)	129.000
10.	Chlorides (Mg/L)	4373.110
11.	Phosphates (Mg/L)	2051.980
12.	Sulphates (Mg/L)	65667.000
13.	Bicarbonates (Mg/L)	7200.000

NB: All values are multiplied by dilution factor of 150 (i.e ×150)



Values expressed in Mean ± S.E.M of 8 determinations

Figure 1.0: Effect of crude oil contaminated feeds on mean body weight indices supplemented with antioxidant in wistar rats.

Control = 0% contamination and No treatment (Control group)

Crude oil = 5% contamination and no treatment (Negative control group)

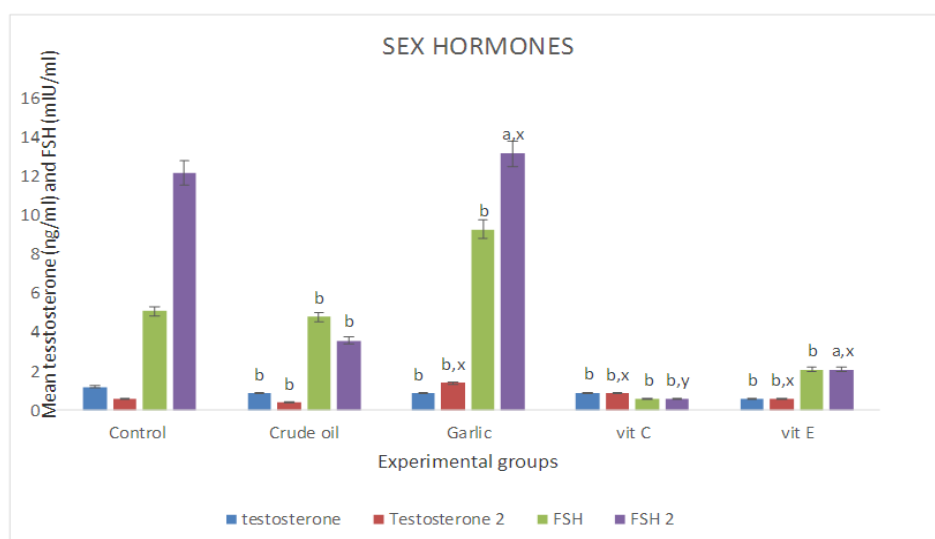
Garlic = 5% contamination and garlic treatment

Vit C = 5% contamination and vitamin C treatment

Vit E = 5% contamination and vitamin E treatment

a- Indicate significant increase compared with the control

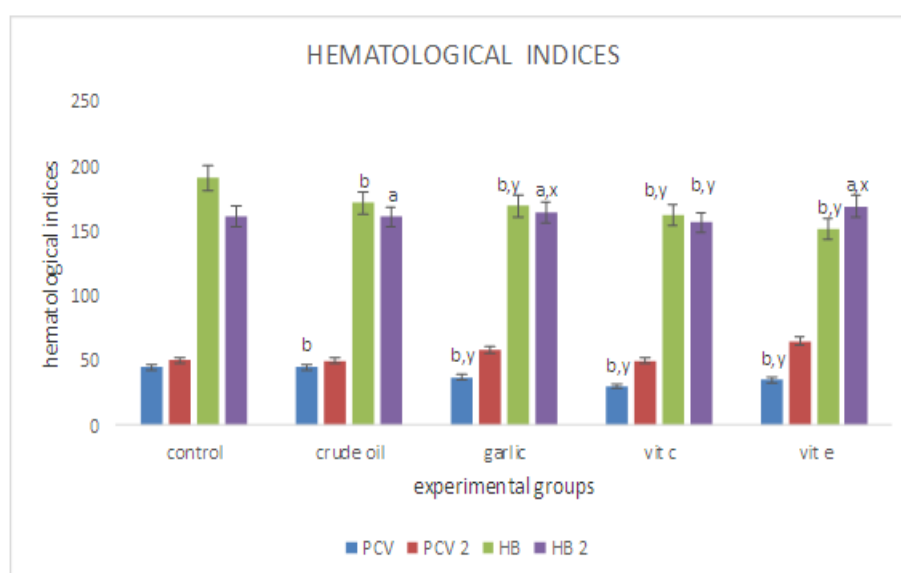
b- Indicate significant decrease compared with the control



Values expressed in Mean ± S.E.M of 8 determinations

Figure 2.0: Effect of crude oil contaminated feeds on (sex hormones) supplemented with antioxidant in wistar rats.

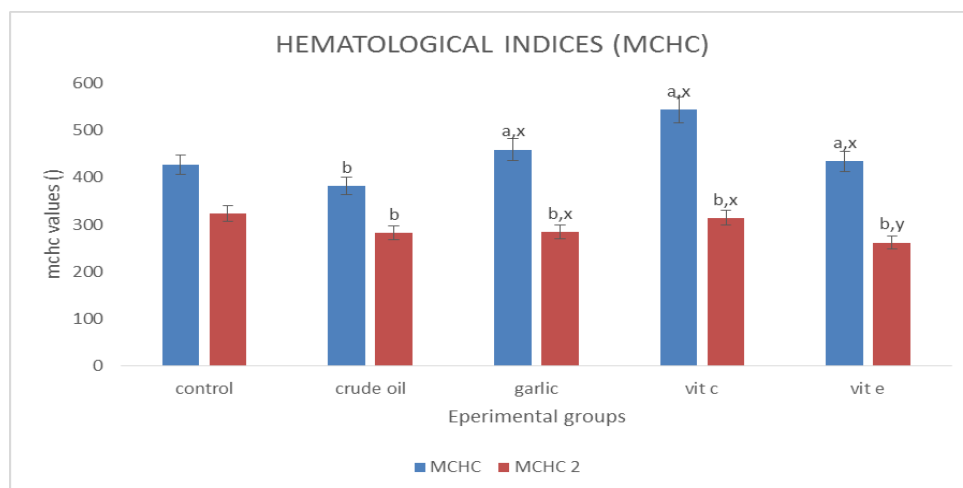
- Control** = 0% contamination and No treatment (Control group)
Crude oil = 5% contamination and no treatment (Negative control group)
Garlic = 5% contamination and garlic treatment
Vit C = 5% contamination and vitamin C treatment
Vit E = 5% contamination and vitamin E treatment
- a-** Significant increase compared with the control
b- Significant decrease compared with the control
x- Significant increase compared with the crude oil control
y- Significant decrease compared with the crude oil control



Values expressed in Mean \pm S.E.M of 8 determinations

Figure 3.0: Effect of crude oil contaminated feeds on ((PCV and HB) supplemented with antioxidant in wistar rats.

- Control** = 0% contamination and No treatment (Control group)
Crude oil = 5% contamination and no treatment (Negative control group)
Garlic = 5% contamination and garlic treatment
Vit C = 5% contamination and vitamin C treatment
Vit E = 5% contamination and vitamin E treatment
- a-** Significant increase compared with the control
b- Significant decrease compared with the control
c- Significant increase compared with the crude oil control
z- Significant decrease compared with the crude oil control



Values expressed in Mean ± S.E.M of 8 determinations

Figure 5.0: Effect of crude oil contaminated feeds on (MCHC) supplemented with antioxidant in wistar rats.

Control = 0% contamination and No treatment (Control group)

Crude oil = 5% contamination and no treatment (Negative control group)

Garlic = 5% contamination and garlic treatment

Vit. C = 5% contamination and vitamin C treatment

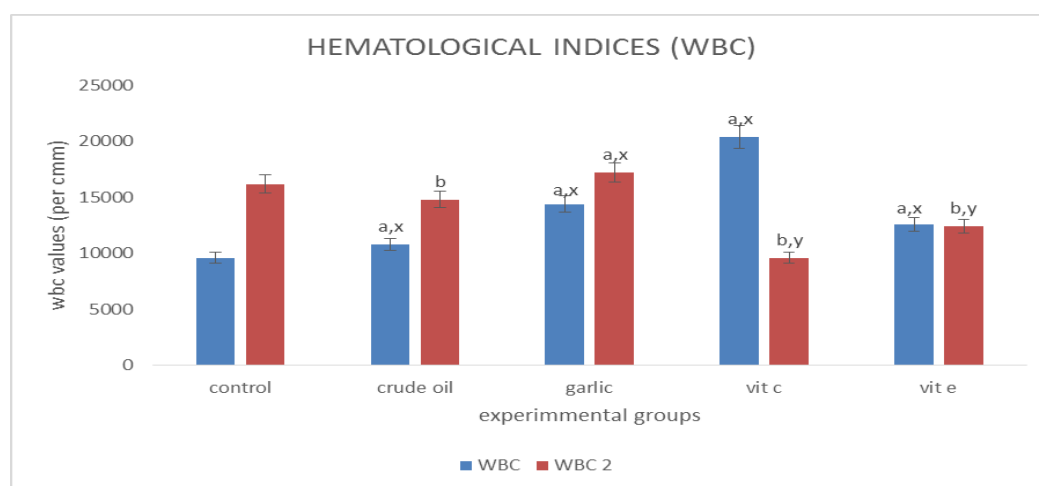
Vit. E = 5% contamination and vitamin E treatment

a- Significant increase compared with the control

b- Significant decrease compared with the control

c- Significant increase compared with the crude oil control

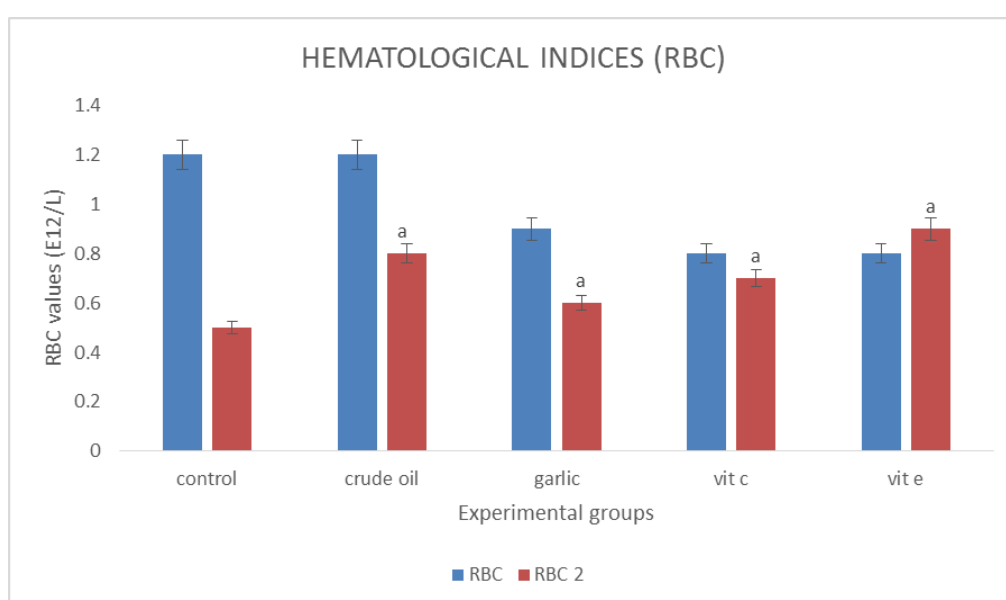
y- Significant decrease compared with the crude oil control



Values expressed in Mean ± S.E.M of 8 determinations

Figure 5.0: Effect of crude oil contaminated feeds on (WBC) supplemented with antioxidant in wistar rats.

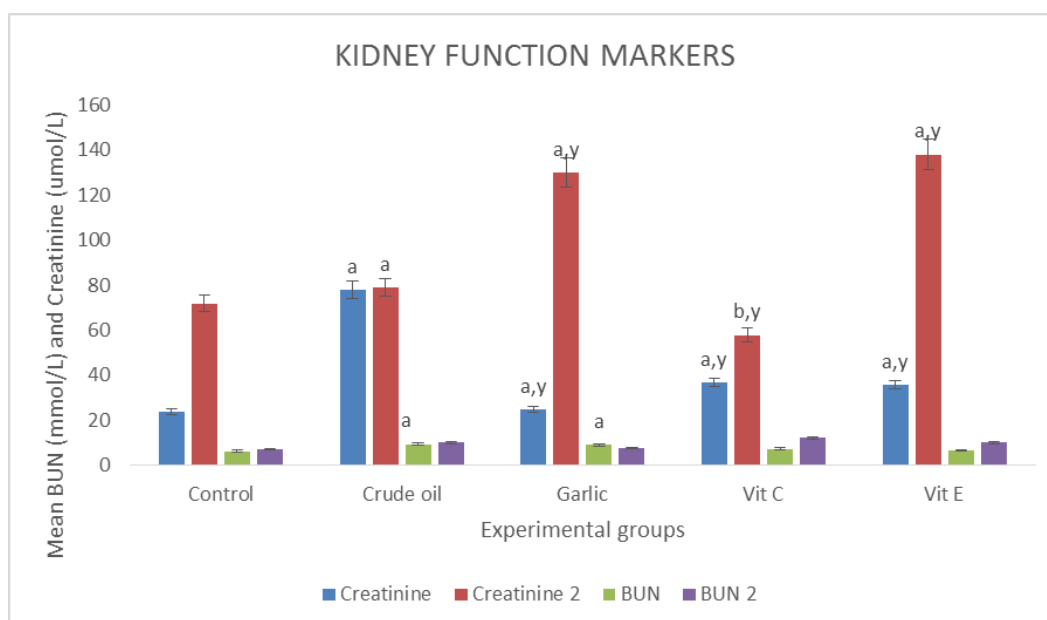
- Control = 0% contamination and No treatment (Control group)**
Crude oil = 5% contamination and no treatment (Negative control group)
Garlic = 5% contamination and garlic treatment
Vit C = 5% contamination and vitamin C treatment
Vit E = 5% contamination and vitamin E treatment
- d- Significant increase compared with the control**
e- Significant decrease compared with the control
f- Significant increase compared with the crude oil control
z- Significant decrease compared with the crude oil control



Values expressed in Mean \pm S.E.M of 8 determinations

Figure 6.0: Effect of crude oil contaminated feeds on Red Blood Cell supplemented with antioxidants in wistar rats.

- Control = 0% contamination and No treatment (Control group)**
Crude oil = 5% contamination and no treatment (Negative control group)
Garlic = 5% contamination and garlic treatment
Vit C = 5% contamination and vitamin C treatment
Vit E = 5% contamination and vitamin E treatment
- a- Indicate significant increase compared with the control**
b- Indicate significant decrease compared with the control



Values expressed in Mean \pm S.E.M of 8 determinations

Figure 7.0: Effect of crude oil contaminated feeds on the kidney function markers supplemented with antioxidants in wistar rats.

Control = 0% contamination and No treatment (Control group)

Crude oil = 5% contamination and no treatment (Negative control group)

Garlic = 5% contamination and garlic treatment

Vit C = 5% contamination and vitamin C treatment

Vit E = 5% contamination and vitamin E treatment

a- Significant increase compared with the control

b- Significant decrease compared with the control

x- Significant increase compared with the crude oil control

y- Significant decrease compared with the crude oil control

DISCUSSION

Crude oil being a major pollutant has been seen to have unfavorable effects in wistar rats. Exposure of humans and animals to crude oil, which is increasing in terms of the environmental pollution and application to body, may present a health problem. In several organs, mainly heart and liver, cell damage is followed by increased levels of a number of cytoplasmic enzymes in the blood, a phenomenon that provides the basis for clinical diagnosis of heart and liver diseases (Ogara, 2008). It is also reported that crude oil consumption can induce a significant decrease in body weight organs (Alonso-Alvarez and Ferrer, 2001).

It was not surprising when the rats in this study showed visible signs of Nigerian Bonny Light Crude Oil (NBLCO) toxicity, which included anorexia and weakness, which corroborate a similar report on mussels where xenobiotics including polycyclic aromatic hydrocarbon (PAH) were reported to significantly reduce the feeding rate of mussels (Di Toro *et al.*, 2000). These observed physical signs could probably have been due to damage and depletion of bodily proteins as well as interference with protein synthesis by free radicals generated by NBLCO. That such damages could be associated with oxidant radical species generated by NBLCO during the course of its metabolism is supported by the fact that antioxidant therapy (Vitamin E or C) significantly corrected these abnormalities in body weight and the amount of food consumed (Ita *et al.*, 2014). It can be speculated that polyaromatic hydrocarbons (PAHs) in crude oil in addition to its other constituents can as well affect the physiologic parameters that are directly related to the nutrient status of an organism. It has been demonstrated in this study that direct oral ingestion of crude oil (NBLCO) could reduce food intake, body weight and cause hypoglycemia. And the hazardous consequences of ingestion of NBLCO could be ameliorated with antioxidant Garlic, vitamin C or E supplementation (Ita *et al.*, 2014).

Nigerian Bonny Light crude oil significantly reduced food consumption and body weight in the treatment rats throughout the experimentation. Crude petroleum contamination can have severe consequences on the health of organisms including humans. Stress, fright, hypothermia and exhaustion are some physical manifestations that may be associated with oil spills (Briggs *et al.*, 1996).

FSH levels are the most important endocrine parameters to evaluate testicular function (Bergmann *et al.*; 1999). In this study, Testosterone and FSH levels were significantly higher in Garlic, Vitamin E and Vitamin C treatment groups while Testosterone and FSH levels were significantly lower ($P < 0.05$) in crude oil control group. This may suggest that BLCO may have adversely affected Leydig and Sertoli cells inhibiting spermatogenesis which may have resulted in subsequent inhibition of Testosterone synthesis resulting in stimulation of LH and FSH secretion. This study is in line with (Obidike *et al.*, 2007) who reported the presence of interstitial exudates, degeneration and necrosis of spermatogenic and interstitial (Leydig) cells in the testis of rats exposed to Nigerian Qua Iboe Brent crude oil. Administration of BLCO showed significant alterations in the hormonal profiles seen in the results of the serum levels of FSH and Testosterone, implying that BLCO possibly adversely

affects the reproductive hormones secretion and the effect may be at the level of the testis. This study further showed that antioxidants have the ability to ameliorate the negative effect of the BLCO.

Hematological parameters have often been associated with health indices and are of diagnostic significance in routine clinical evaluation of the state of health (Patrick-Iwuanyanwu *et al.*, 2007). This study has demonstrated that long term exposure to petroleum samples particularly kerosene and petrol induces anemia. The resulting anemia is in accordance with the report of Krishan and Veena (1980), which showed the suppressive effect of petroleum products on erythropoiesis. Leighton *et al.*, (1985) characterized hemolytic anemia as hallmark of oil toxicity in animals. Also, studies have shown that the toxic components of crude oil changes blood chemistry and induce anaemia by causing bone marrow hypoplasia and interfered with platelet production in the animal, hence the reduced values of Hb and PCV. Similarly, altered blood parameters have been reported in other forms of life exposed to petroleum products, Lutcavage *et al.*, (1995) demonstrated the toxicity of petroleum in different species of birds, which showed significantly reduced red blood cell counts and red blood cell polychromasia with inherent regenerative anemia. The ability of the treatment antioxidants to ameliorate the anemic condition was also observed.

In the kidney function markers, crude oil increased the level of blood urea nitrogen, which the antioxidants further increased to show kidney damage.

CONCLUSION

On the basis of the findings from this work and correlation with other works, it is evident that hydrocarbons found in crude oil influence, the physiology, health, growth and reproductive potentials of subjects upon exposure. The observed physiological findings are also pointers to the toxicity of crude oil. The possible mechanism of crude oil toxicity was found to be elaborated in the anemic condition observed, reduction in sex hormonal level, general body weight and kidney function enzymes. Also, Antioxidants such as Vitamin C, Vitamin E and Garlic are also very useful in ameliorating these toxic effects caused by crude oil.

Competing interest

The authors affirm that there is no conflict of interest in the publication.

Ethical approval

All authors hereby declare that the research has been determined exempt from review by the university animal research or ethics review committee and that the principles of laboratory animals were strictly observed.

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