



## Evaluation of the Protective Effects of Bitter Leaf (*Vernonia amygdalina*) on Haematological Indices of Rats Fed with Crude Oil Treated Diet

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**ABSTRACT:** A lot of researches are ongoing on the use of plant to ameliorate the toxicity of various toxicants. This study evaluated the protective effects of bitter leaf (*Vernonia amygdalina*) on haematological indices of rats fed with crude oil treated diet. Twenty four male albino Wister rats with weight range of 200.75g – 217.54g were used for the experiment and were randomly assigned to six groups: group A: Control; group B: Fed with 100g of feed + 5.0g of bitter leaf; group C: Fed with 100g of feed + 10.0g of bitter leaf; group D: Fed with 100g of feed + 10g of bitter leaf + 4.0ml of crude oil; group E Fed with 100g of feed + 5.0g of bitter leaf + 4.0ml of crude oil; group F: Fed with 100g of feed + 4ml of crude oil. The results showed that treatment of diets with bitter leaf minimized crude oil toxicity, as red blood cell count, haemoglobin concentration; hematocrit values and white blood cell indices were maintained close of the control values. This study indicates that intake of bitter leaf reduced the toxic effect of crude oil treated diet on animals. Therefore, consumption of bitter leaf should be encouraged among the inhabitants of crude oil bearing communities of the world who are exposed to crude oil contaminated food and water.

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In the Niger Delta area of Nigeria some rural dwellers are exposed to crude oil because they use the chemical in various forms to treat a variety of ailments. They do this either by ingesting the crude oil or taken in combination with other substances (Dienye, 2012). In addition, humans get exposed to crude oil by consuming contaminated food, either directly or through the food chain (Sunmonu and Oloyode, 2007). Crude oil is injurious to animal's health (Achuba and Ogwumu 2014a; Achuba Nwokogba 2015a), which can be acute or chronic. Acute exposure of animals to crude oil usually result in eye irritation, nausea, vomiting, diarrhea and confusion, while chronic effects of petroleum hydrocarbon include decreased immune function, organ damage, biochemical and physiological abnormalities. (Unwin *et al.*, 2006; Tormoehlen *et al.*, 2014; Achuba and Nwokogba, 2015b; Achuba *et al.* 2016). In fact, petroleum hydrocarbon causes metabolic imbalances in experimental animals (Achuba *et al.*, 2016). In addition, crude oil had been implicated in the alteration of haematological parameters in animal models (Ita *et al.*, 2011; Ita *et al.*, 2013; Achuba and Nwokogba 2015a). Moreover, researchers have shown that antioxidants such as vitamins (Achuba and Otuya, 2006), palm oil (Achuba and Ogwumu, 2014b), honey (Achuba and Nwokogba, 2015ab) and *Moringa oleifera* (Ujah *et al.*, 2013; Achuba *et al.*, 2016) can

be used to attenuate petroleum hydrocarbon toxicity in animals.

Bitter leaf (*Vernonia amygdalina*) is an important medicinal plant which has hypoglycemic, anti-diabetic and anticholesterol properties (Kigigha, *et al.*, 2015; Owen *et al.*, 2011). Moreover, the extract has been found to mitigate chemical toxicity (Ikeh *et al.*, 2014). However, information on the use of bitter leaf as an antidote for toxicity of crude oil tainted diets in rats is scanty. Therefore, the aim of this study was to evaluate the protective potency of *Vernonia amygdalina* treated diet against crude oil toxicity

### MATERIALS AND METHODS

Bitter leaf was obtained from a local farm in Abraka. Identification was performed by the Department of Botany, Delta State University, Abraka, Nigeria. A local animal dealer supplied the experimental rats. Supply of crude oil was done by Nigerian National Petroleum Corporation (NNPC), Port Harcourt, Nigeria.

*Experimental Design:* Twenty four rats (average weight 200.75g – 217.54g and age 3-4 months) and assigned into groups in different compartments, each containing four rats. They were fed with just growers mash and clean water throughout the one week of

acclimatization. After acclimatization, each group was subjected to different mixture of feed as group A for Control; group B those fed with 100g of feed + 5.0g of bitter leaf, group C those fed with 100g of feed +10.0g of bitter leaf; group D for those fed with 100g of feed + 10g of bitter leaf + 4.0ml of crude oil; group E for those fed with 100g of feed + 5.0g of bitter leaf + 4.0ml of crude oil and group F for those fed with 100g of feed + 4ml of crude oil.

The rats were exposed to the diets for thirty days and had free access to clean drinking water.

**Determination Haemoglobin Concentration and Red Cell Indices:** After thirty days blood samples were collected from each of the groups through heart puncture and used to determine hematological indices using standard procedures. The concentration of haemoglobin and Packed Cell Volume were determined as reported by Achuba and Ogwumu (2014a). The Mean cell volume (MCV), the mean cell haemoglobin (MCH) and the MCHC were determined according to methods reported by Achuba *et al.*, (2016). Neuburger rule counting chamber (Haemocytometer) was used to estimate the white blood cell count (WBC).

## RESULTS AND DISCUSSION

Crude oil is a potential industrial, domestic and environmental toxicant (Onwurah *et al.*, 2013; Oguwike *et al.*, 2014; Rostami and Soltan, 2016; Adienbo and Nwafor, 2010). This is the basis for the toxicity observed in this investigation (Table 1), which is in tandem with earlier studies (Achuba and Ahwin, 2009; Achuba and Ogwumu, 2014a; Achuba and Nwokogba, 2015). It is reported that reduction in haematological index is an indication of anemic state of an animal (Christain *et al.*, 2016). In addition, the increase in WBC indicates response of the immune system to disease stimulating toxicants (Ita *et al.*, 2013; Da Silva Júnior *et al.*, 2013; Okoye *et al.*, 2014). This explains why there is alteration in immune cells in this study (Table 1), which agrees with the report of Okonkwo *et al.* (2016). Previous studies indicated that certain organic and inorganic substances can mitigate the toxic effect of petroleum hydrocarbon (Achuba and Nwokogba, 2015b; Achuba *et al.*, 2016). This is consistent with the result of the present study. The addition of bitter leaf to diets pretreated with crude oil reduced the deleterious effect of hydrocarbon on the exposed animals. In fact, the wound healing property of bitter leaf is previously documented (Oguwike *et al.*, 2014).

**Table 1:** Effect of bitter leaf on hematological indices of rats fed with hydrocarbon treated diet

	A	B	C	D	E	F
RBC( $10^3/\mu\text{l}$ )	7.93 ± 0.97 <sup>a</sup>	8.45±0.43 <sup>a</sup>	7.32±0.49 <sup>a</sup>	7.84±0.49 <sup>a</sup>	7.69±0.18 <sup>a</sup>	7.01±0.27 <sup>b</sup>
HGB (g/dl)	14.68± 0.39 <sup>a</sup>	14.57±0.43 <sup>a</sup>	14.35±0.61 <sup>a</sup>	13.41±0.88 <sup>b</sup>	13.20±0.20 <sup>b</sup>	13.18±0.27 <sup>b</sup>
HCT (%)	44.68±0.39 <sup>a</sup>	42.43±1.45 <sup>b</sup>	42.10±0.80 <sup>b</sup>	40.73±1.16 <sup>b</sup>	39.40±0.70 <sup>b</sup>	37.25±0.50 <sup>b</sup>
WBC ( $10^3/\mu\text{l}$ )	6.35 ± 0.48 <sup>a</sup>	4.30± 0.29 <sup>b</sup>	5.53±0.36 <sup>b</sup>	5.85± 0.31 <sup>a</sup>	3.17± 0.38 <sup>b</sup>	2.23± 0.22 <sup>b</sup>
MON (%)	1.45 ± 0.13 <sup>a</sup>	5.55 ± 0.21 <sup>b</sup>	0.80± 0.18 <sup>b</sup>	0.73±0.10 <sup>b</sup>	0.87± 0.06 <sup>b</sup>	0.40 ±0.08 <sup>b</sup>
NEU (%)	16.05±0.93 <sup>a</sup>	12.03±0.28 <sup>b</sup>	18.23±0.65 <sup>b</sup>	12.33±0.60 <sup>b</sup>	11.63± 0.85 <sup>b</sup>	13.28±0.62 <sup>b</sup>
EOS (%)	1.15±0.13 <sup>a</sup>	1.18±0.13 <sup>a</sup>	1.63±0.21 <sup>b</sup>	3.18±0.25 <sup>b</sup>	2.77±0.15 <sup>b</sup>	2.27±0.25 <sup>b</sup>
BAS (%)	3.05 ± 0.13 <sup>a</sup>	13.18±0.33 <sup>b</sup>	3.35±0.39 <sup>a</sup>	4.65±0.56 <sup>b</sup>	6.46±0.49 <sup>b</sup>	7.90±0.22 <sup>b</sup>
LYM ( $10^3/\mu\text{l}$ )	5.15±0.26 <sup>a</sup>	2.95±0.13 <sup>b</sup>	4.30±0.37 <sup>b</sup>	4.45±0.39 <sup>b</sup>	2.33 ±0.25 <sup>b</sup>	1.55±0.22 <sup>b</sup>
PLT ( $10^3/\mu\text{l}$ )	464.0±12.91 <sup>a</sup>	632.25±7.41 <sup>b</sup>	537.25±8.77 <sup>b</sup>	376.0±8.60 <sup>b</sup>	228.33±4.93 <sup>b</sup>	270.75±1.71 <sup>b</sup>
MCV ( $\mu\text{M}^3$ )	58.55±0.58 <sup>a</sup>	36.15±24.77 <sup>a</sup>	53.30±1.26 <sup>a</sup>	47.70±0.84 <sup>a</sup>	50.20±1.21 <sup>a</sup>	47.95±5.38 <sup>a</sup>
MCH (pg)	19.03±0.26 <sup>a</sup>	17.15±0.21 <sup>b</sup>	18.13±1.03 <sup>a</sup>	15.90±1.22 <sup>b</sup>	17.07±0.15 <sup>b</sup>	17.55±0.45 <sup>b</sup>
MCHC (g/dl)	32.53±0.51 <sup>a</sup>	33.28±0.57 <sup>a</sup>	35.95±0.97 <sup>b</sup>	33.70±0.84 <sup>a</sup>	33.50±0.79 <sup>a</sup>	34.18±1.43 <sup>a</sup>
RDW (%)	17.60±0.83 <sup>a</sup>	17.25±0.29 <sup>a</sup>	16.80±0.46 <sup>a</sup>	16.53±0.59 <sup>a</sup>	17.10±0.20 <sup>a</sup>	17.05±0.13 <sup>a</sup>

Values are expressed as means ± SEM of four animals in each group. a, b, c Significantly different from control,  $P < 0.05$ .

An acute onset of disease process increases RDW and reduces the number of eosinophils, monocytes, basophils and lymphocytes (Förhéczy *et al.*, 2009). It is no surprise that exposure of subjects to petroleum hydrocarbon could elicit disease process (Ita *et al.*, 2015). This agrees with the present investigation that showed alterations in white blood cell profile (Table 1).

Similarly, inculcation of ground bitter leaf into the contaminated diets before feeding the rats maintained white blood cell types relative to the level in control animals. However, the white blood types were enhanced by bitter leaf in diet close to values in control

rats. The immunostimulant property of bitter leaf was earlier reported (Osho *et al.*, 2014).

This study was able to establish that intake of bitter leaf ameliorated haematotoxicity of petroleum hydrocarbon treated diet on exposed animals. Therefore, intake of bitter leaf should be encouraged among the inhabitants of crude oil bearing communities of the world.

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