#### **Research Article**

# Palm Oil Fractions Alter Acute Cadmium Mediated Haematotoxicity

Samuel Ogheneovo Asagba<sup>1</sup>, Patrick Chukwuyenum Ichipi-Ifukor<sup>1\*</sup>, Rita Ngozi Ichipi-Ifukor<sup>2</sup>, John Chukwuma Oyem<sup>3</sup>

#### **Abstract**

**The objective** of the research was to contribute to the continual search for natural products that could mitigate alterations in haematological indices due to cadmium poisoning.

**Materials and methods.** Seventy-two male Wistar rats were mobilized and divided into six groups (A-F). Group A served as the control one which was neither exposed to cadmium nor treated with palm oil fraction. Group B was not treated with palm oil fractions but received a single dose of 20mgkg<sup>-1</sup> body weight of cadmium chloride solution on the 29<sup>th</sup> day of the experiment, while Groups C-E received 5mlKg<sup>-1</sup> bodyweight of crude palm oil, silica gel extract, bleached extract and unsaponifiable extract of palm oil respectively for 28 days before a single dose of 20mgKg<sup>-1</sup> body weight of cadmium chloride on the 29<sup>th</sup> day. Blood samples were collected from four animals via cardiac puncture on the 29<sup>th</sup>, 30<sup>th</sup> and 31<sup>st</sup> day within intervals of 12h, 24h and 48h after cadmium administration. This was then analysed for haematological parameters using an automated haematological analyser. Data analysis was carried out using the one-way factor analysis of variance at a significant level of p<0.05.

**Results.** The impact of cadmium intoxication on haematological indices in rats was time-dependent and was mostly felt at the end of the 48-hour period indicating a significant decrease in the packed cell volume, haemoglobin and white blood cells values, while the increase due to cadmium was observed in mean corpuscular haemoglobin and mean corpuscular volume values; pre-treatment of palm oil fractions mitigated the noxious effects of cadmium significantly near control values.

**Conclusions.** Crude palm oil and its fractions have the ability to mobilize antioxidant defence potentials against cadmium damage to blood cells.

#### **Keywords**

cadmium; palm oil; alter; toxicity; haematology; haematotoxicity

- <sup>1</sup>Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Nigeria
- <sup>2</sup>Department of Biochemistry, Faculty of Life Sciences, University of Benin, Nigeria
- <sup>3</sup>Department of Anatomy Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria
- \*Corresponding author: patychykyresearch@hotmail.com



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# Problem statement and analysis of the latest research

Heavy metals are a class of chemicals known for their very risky and dangerous nature as slight carelessness in their handling could be indicative of very significant fatal experiences [1, 2]. Cadmium which belongs to a class of these chemicals is said to be one of the most dangerous of them as it bio accumulates [3, 4] when it eventually builds up in high concentrations, contributes significantly to the gradual shut down and deterioration of most biological systems due to the high affinity it has for the liver and kidney [5, 6]. The mechanism of cadmium toxicity has been reported to involve reduction in the functionality of cell membrane binding to the ion transporter metallothionein, as well as the glutathione cascade which eventually affects the maintenance of cell membrane integrity and the inhibition of oxidative stress [7, 8].

The use of several physiological indicators in monitoring responses to changes in environment and chemical insult has been well established in several studies [9, 10]. Such indicators which also include hematological indices are said to be the first line of indicators for establishing pathological changes and the health status of those who have been exposed to chronic or acute toxicities of certain compounds [11]. Since blood remains the most important of all tissues in biological systems, it remains significant for stable metabolic process as it is the only fluid which is available for transport of nutrients to many other parts of the body [12, 13, 14].

Substances rich in Phytonutrients are increasingly being used as suitable alternatives for the management of metal toxicity [15, 16, 17, 18, 19, 20, 21, 22]. Palm oil is said to be majorly made up of fatty acids, sterols and certain other components such as tocopherol and the carotenoids [23, 24, 25]. They also contain certain rich phospholipids that have remained essential in the chelating of heavy metals and remain significant contributors to hormonal balance and improve easy transport and absorption of food nutrients [26]. This report is a continuation of an earlier report by Ichipi-Ifukor PC et al. [27, 28] on the role of rich phytonutrients in several fractions of palm oil in mitigation of several indices of acute cadmium toxicity.

The objective of the research was to focus on the level of protection conferred by palm oil against haematotoxicity in rats during acute cadmium poisoning as a result of pre-treatment with crude palm oil (CPO), silica gel fraction, bleached fraction and unsaponifiable fractions of palm oil.

#### **Ethical Considerations**

Research approval was obtained from the Delta State University Faculty of Science Ethics Committee; the approval number ETH/15/16/PG223056

was assigned. This was in accordance to the guidelines and declarations of Animal Research Ethics and the World Medical Association [29, 30] on animal use in biomedical research.

#### 1. Materials and Methods

#### **Extraction of Palm Oil.**

Extraction of oil was carried out after purchasing oil palm fruits from a farm at Obiaruku Delta State, Nigeria. The identification was carried out at the Department of Botany of the Delta State University, Abraka where it was authenticated Tenerra specie with the herbarium number ID/2017/16807/Tenerra spp. assigned. The extraction process was preceded by washing 10 g of the fruits and immediately followed by a four-hour boiling process. The pulp extraction was carried out by pounding the well-cooked fruits in a mortar and transferred to a bowl with 101 of water and stirred thoroughly. The separation of the fibers was done using a sieve while the filtrate was immediately placed on a cooking pot and allowed to boil under a regulated temperature of 150°C for the next five hours. Two liters of water were sprinkled on the boiled mixture after a period of 30 minutes, sprinkling at the surface. The palm oil was then scooped at the top and heated slightly to allow the evaporation of any traces of water in the scooped oil.

#### Fractionation of CPO and Animal Treatment.

The details of methods for the fractionation of the three palm oil fractions (unsaponifiable extract (UPE), silica gel extract (SGE) and bleached extract) have been earlier published Twumasi P et al. and Ichipi-Ifukor PC et al. [16, 27]. Animal treatment, on the other hand, followed the same experimental design reported by Ichipi-Ifukor PC et al. [27, 28]; thus, 72 male Wistar rats were mobilized for the study following standard protocols for animal care as recommended by Animal Research Ethics [29]. Group A served as the control one which was neither exposed to cadmium nor treated with palm oil fraction. Group B was not treated with palm oil fractions but received a single dose of 20mgkg<sup>-1</sup> body weight of cadmium

chloride solution on the 29<sup>th</sup> day of the experiment, while Groups C-E received 5mlKg<sup>-1</sup> bodyweight of CPO, SGE, bleached extract (BE) and UPE of palm oil respectively for 28 days before a single dose of 20mgKg<sup>-1</sup> body weight of cadmium chloride on the 29<sup>th</sup> day.

## Collection of Blood and Haematological Analysis.

Blood samples were collected from four animals via cardiac puncture on the 29<sup>th</sup>, 30<sup>th</sup> and 31<sup>st</sup> day within intervals of 12h, 24h and 48h after cadmium administration. This was then analysed for haematological parameters using an automated haematological analyser (produced by Shenzhen Mindray Bio-Medical Electronics Co. Ltd., China).

#### Method of Statistical Analysis.

Data analysis was carried out using the statistical package for the social sciences, version 21 (SPSS 21) computer software. The determination of significant difference was done using the one-way factor analysis of variance (ANOVA). Mean difference across groups was further compared using the Tukey HSD analysis at a significant level of p<0.05

#### 2. Results and Discussion

The mechanism of the deleterious effects of cadmium is reported to be time and concentration dependent and has been severally reported to take a toll on the body physiologic conditions. The observed non-significant alterations in the packed cell volume (PCV), haemoglobin (Hb), mean corpuscular haemoglobin concentration (MCHC), red blood cells (RBC) and white blood cells (WBC) across all groups at the end of the first period of cadmium postadministration may be a result of the time frame and metabolism of cadmium before it is able to cause its deleterious effects [31, 32]. Observed changes occasioned by increase in platelet counts, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) values in rats given only cadmium and those pre-treated with SGE may be indicative of early onset of macrocytic anaemia which occurs as a result of low absorption of folic acid

and is reflected in increased MCH, while increased MCV is said to be indicative of vitamin deficiency and anaemia [33, 34]. Although these changes observed 12 hours after cadmium administration may not have occurred as a result of cadmium poisoning but possibly as a result of other physiologic conditions (Table 1).

The trend of the result after the second period of post-cadmium intoxication may have implicating inferences on the haematotoxic effects of cadmium as reduced platelet and Hb counts were reported in rats given cadmium only. Reduced blood haemoglobin is indicative of disruption of erythrocytes as cadmium has been reported to contribute significantly to the destruction of erythrocytes via lipid peroxidation occasioned by free radical generation [35, 36, 37]. Based on the result obtained, it could be inferred that palm oil fraction pre-treatment conferred on other groups (C-F) some levels of protection against erythrocyte destruction as their Hb counts were at the levels similar to those of the control group. This study indicated consequential increase in MCH and MCV of rats that were given only cadmium (Group B) and CPO (Group C) at the end of 24-hour cadmium intoxication similar to that of after the 12-hour period and confirmed our earlier inference of a possible onset of macrocytic anaemia. The confirmation of the statements made here is given by the reduced levels of WBC in rats treated with cadmium only as compared to the control group and eventual increase in rats pre-treated with palm oil fraction. A WBC count is said to be a notable indicator of immune responses against several physiologic disorders; thus, their increased mobilization in Groups C-F is indicative of responses against cadmium insult [38, 39, 40]. The eventual drop in WBC in the group treated with cadmium only may be said to be indicative of autoimmune response to cadmium which may have implicated the increase in erythrocyte cells that necessitated the rise in MCH and MCV values due to the inability of RBC to transport oxygen [41, 42, 43]. Cadmium, being a competitive metal to the binding of Fe in erythrocytes, has been previously implicated for its contribution to reduced oxygen transport within the body fluid [44, 45]; hence this exchange may have

<b>Table 1.</b> Effects of Palm Oil and Palm Oil Fractions on Haematological Indices 12 Hours after Cadmium
Administration

	PCV (%)	Platelet (x 10 <sup>9</sup> /L)	Hb (g/L)	MCH (pg/cell)	MCHC (%)	MCV (fl)	RBC (x10 <sup>12</sup> /L)	WBC (x 10 <sup>9</sup> /L)
A	43.00 ±6.27 <sup>a</sup>	110.50 ±9.91 <sup>a</sup>	$14.17 \pm 1.84^{a}$	32.65 ±5.24a <sup>b</sup>	33.55 ±0.44 <sup>a</sup>	98.68 ±7.52 <sup>a</sup>	4.45 ±0.25 <sup>a</sup>	8.43 ±2.61 <sup>a</sup>
В	$38.25 \pm 2.36^{a}$	184.50 ±10.37 <sup>b</sup>	12.00 ±0.96 <sup>a</sup>	39.48 ±5.54 <sup>b</sup>	33.03 ±0.68 <sup>a</sup>	120.48 ±17.52 <sup>b</sup>	3.43 ±0.51 <sup>a</sup>	6.50 ±2.89 <sup>a</sup>
С	44.75	93.00	15.00	35.03	33.23	106.05	4.30	6.10
	±0.50 <sup>a</sup>	±4.76 <sup>a</sup>	±0.82 <sup>a</sup>	±0.94 <sup>ab</sup>	±0.15 <sup>a</sup>	±1.46 <sup>ab</sup>	±0.34 <sup>a</sup>	±4.20 <sup>a</sup>
D	41.75	184.50	13.65	29.45	32.55	92.10	4.38	6.20
	±0.50 <sup>a</sup>	±6.40 <sup>b</sup>	±0.91 <sup>a</sup>	±1.32 <sup>ac</sup>	±1.50 <sup>a</sup>	±2.46 <sup>a</sup>	±0.29 <sup>a</sup>	±1.93 <sup>a</sup>
Е	43.00	113.25	13.88	30.90	32.30	93.28	4.45	9.65
	±2.16 <sup>a</sup>	±13.50 <sup>a</sup>	±1.51 <sup>a</sup>	±2.20 <sup>ac</sup>	±1.46 <sup>a</sup>	±6.35 <sup>a</sup>	±0.31 <sup>a</sup>	±0.34 <sup>a</sup>
F	39.50	97.50	12.55	27.60	32.55	84.37	4.50	7.45
	±1.00 <sup>a</sup>	±9.57 <sup>a</sup>	±1.50 <sup>a</sup>	±2.21 <sup>ac</sup>	±1.50 <sup>a</sup>	±4.62 <sup>a</sup>	±0.38 <sup>a</sup>	±5.72 <sup>a</sup>

*Notes:* All values are presented as Mean  $\pm$ SD of four replicates. Values followed by different alphabet superscripts on the same column indicate a significant difference.

**Key:** A = Control; B = Cadmium only; C = CPO + Cadmium; D = SGE + Cadmium; E = BE + Cadmium; F = UPE + Cadmium

necessitated the enhancement of WBC destruction which are important for boosting immunity (Table 2).

The time-dependent noxiousness due to cadmium was notably manifested in reduction of the PCV, Hb, MCV and WBC values (Table 3) after 48 hours in cadmium only group (B) as compared to the control group (A) which is in tandem with the trend reported on effect of cadmium administration on activities of aminotransferases, oxidative enzymes and lipid profile of rats [27, 32, 46]. Although comparable to other groups, the PCV reduced in Group C, but did not change in Groups D-F as compared to the control one; Hb remained glaringly unchanged; MCV values reduced in all other groups as compared to the control group; WBC counts in Groups C, D and F did not change but reduced remarkably in Group E as compared to all other groups, save for cadmium only group, which also gives credence to the earlier

claims of the ability of cadmium to induce autoimmune responses that may necessitate destruction of WBC over time. Also, in accordance with the ability of cadmium to have a negative effect on haematological parameters, there were studies by Ognjanović BI et al. [47], Asagba SO [48], Houkpatin ASY et al. [49], Parekh HM, Tank SK [36], who reported that cadmium gave rise to destruction of WBC, reduced PCV and HB and finally anaemia in rats.

Conspicuous changes in platelet count were seen in Group D only, while MCHC remained unchanged across all experimental groups as compared to the control one. MCH values were observed to have increased remarkably in Group E as compared to Group B, while it remained unchanged as compared to all other groups (A, C, D, F). This observation may have been a result of the combined negative effect of bleached palm oil and cadmium which was exerted on the animal necessitating in-

<b>Table 2.</b> Effects of Palm Oil and Palm Oil Fractions on Haematological Indices 24 Hours after Cadmium
Administration

	PCV (%)	Platelet (x 10 <sup>9</sup> /L)	Hb (g/L)	MCH (pg/cell)	MCHC (%)	MCV (fl)	RBC (x10 <sup>12</sup> /L)	WBC (x 10 <sup>9</sup> /L)
A	47.50 ±5.00 <sup>a</sup>	102.70 ±2.22 <sup>a</sup>	15.30 ±1.43 <sup>a</sup>	29.45 ±8.50 <sup>a</sup>	32.80 ±0.58 <sup>a</sup>	91.50 ±0.69 <sup>a</sup>	$4.40 \pm 0.00^{a}$	4.00 ±0.00 <sup>a</sup>
В	38.00 ±4.76 <sup>a</sup>	93.00 ±4.76 <sup>b</sup>	$11.70 \pm 0.00^{b}$	42.60 ±2.30 <sup>b</sup>	33.40 ±0.00 <sup>a</sup>	128.63 ±5.32 <sup>b</sup>	$4.10 \pm 0.20^{a}$	4.01 ±0.00 <sup>a</sup>
С	43.75 ±2.50 <sup>a</sup>	164.75 ±23.60°	15.08 ±1.51 <sup>a</sup>	26.57 ±1.96 <sup>a</sup>	$33.35 \\ \pm 0.06^{a}$	81.73 ±3.86 <sup>a</sup>	3.78 ±0.53 <sup>a</sup>	7.23 ±2.45 <sup>ab</sup>
D	37.50 ±8.66 <sup>a</sup>	100.00 ±0.00 <sup>a</sup>	12.50 ±2.89 <sup>ab</sup>	34.10 ±0.92 <sup>a</sup>	33.30 ±0.00 <sup>a</sup>	102.35 ±2.71 <sup>ab</sup>	3.65 ±0.75 <sup>a</sup>	14.05 ±2.25 <sup>b</sup>
Е	41.25 ±2.50 <sup>a</sup>	180.00 ±27.08 <sup>c</sup>	13.72 ±0.85 <sup>ab</sup>	31.90 ±3.93 <sup>a</sup>	33.30 ±0.00 <sup>a</sup>	95.90 ±4.68 <sup>a</sup>	4.33 ±0.29 <sup>a</sup>	8.25 ±2.87 <sup>ab</sup>
F	37.00 ±3.45 <sup>a</sup>	151.00 ±8.87°	12.80 ±1.50 <sup>ab</sup>	30.83 ±1.44 <sup>a</sup>	33.78 ±1.53 <sup>a</sup>	88.35 ±1.74 <sup>a</sup>	4.18 ±0.33 <sup>a</sup>	6.95 ±0.91 <sup>ab</sup>

*Notes:* All values are presented as Mean  $\pm$ SD of four replicates. Values followed by different alphabet superscripts on the same column indicate a significant difference.

**Key:** A = Control; B = Cadmium only; C = CPO + Cadmium; D = SGE + Cadmium; E= BE + Cadmium; F= UPE + Cadmium

hibition of vitamin absorption possibly because of macrocytic anaemia in these sets of rats. Relative to the control group, rising RBC were noted only for Group F but remained unaltered across all other groups. The possible reason for the rise in RBC values 48 hours after cadmium poisoning may be the high availability of carotenoids in unsaponified fractions of palm oil pre-administered to rats in this group. Carotenes were previously said to contribute notably in boosting of RBC production and may have some extent of contribution to the development of the red pigment in the blood [50, 51, 52].

### 3. Conclusions

At the end of 48-hour post cadmium intoxication, the observed reduced influence of cadmium on haematological parameters may have been as a result of the ability of palm oil and its fractions to mobilize antioxidant defence potentials against cadmium which contributed significantly to preventing dam-

age to blood cells.

#### **Declaration**

The authors declare that there is no conflict of interest and that there was no external source of funding or sponsorship for this study.

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	PCV (%)	Platelet (x 10 <sup>9</sup> /L)	Hb (g/L)	MCH (pg/cell)	MCHC (%)	MCV (fl)	RBC (x10 <sup>12</sup> /L)	WBC (x 10 <sup>9</sup> /L)
A	43.75	85.00	14.25	31.78	32.55	119.60	3.73	11.25
	±2.50 <sup>a</sup>	±5.78 <sup>a</sup>	±1.50 <sup>a</sup>	±2.86 <sup>ab</sup>	±1.50 <sup>a</sup>	±19.57 <sup>a</sup>	±0.91 <sup>a</sup>	±2.87 <sup>a</sup>
В	34.25 ±1.50 <sup>b</sup>	97.50 ±2.17 <sup>b</sup>	11.28 ±0.85 <sup>b</sup>	$27.25 \pm 2.46^{a}$	33.20 ±0.00 <sup>a</sup>	81.98 ±7.701 <sup>b</sup>	4.20 ±0.40 <sup>ab</sup>	4.50 ±1.00 <sup>b</sup>
С	35.00	85.00	11.69	27.90	33.35	83.55	4.20	14.45
	±0.00 <sup>b</sup>	±5.77 <sup>a</sup>	±0.02 <sup>ab</sup>	±1.50 <sup>ab</sup>	±0.06 <sup>a</sup>	±4.56 <sup>b</sup>	±0.23 <sup>ab</sup>	±0.52 <sup>a</sup>
D	40.50	182.50	14.23	30.13	34.60	84.38	4.70	12.73
	±1.00 <sup>a</sup>	±13.63°	±1.42 <sup>a</sup>	±4.65 <sup>ab</sup>	±1.67 <sup>a</sup>	±4.62 <sup>b</sup>	±0.20 <sup>ab</sup>	±5.30 <sup>a</sup>
Е	42.25 ±2.63 <sup>a</sup>	82.50 ±5.00 <sup>a</sup>	$14.15 \\ \pm 0.98^{a}$	33.85 ±0.71 <sup>b</sup>	33.22 ±0.15 <sup>a</sup>	93.45 ±4.40 <sup>b</sup>	4.35 ±0.25 <sup>ab</sup>	4.10 ±0.50 <sup>b</sup>
F	42.50	105.0	14.15	29.15	33.30	87.55	4.85	14.50
	±2.89 <sup>a</sup>	±4.41 <sup>b</sup>	±0.98 <sup>a</sup>	±1.67 <sup>ab</sup>	±0.00 <sup>a</sup>	±4.91 <sup>b</sup>	±0.06 <sup>b</sup>	±2.12 <sup>a</sup>

**Table 3.** Effects of Palm Oil and Palm Oil Fractions on Haematological Indices 48 Hours after Cadmium Administration

*Notes:* All values are presented as Mean  $\pm$ SD of four replicates. Values followed by different alphabet superscripts on the same column indicate a significant difference.

**Key:** A = Control; B = Cadmium only; C= CPO + Cadmium; D = SGE + Cadmium; E= BE + Cadmium; F = UPE + Cadmium

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