EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
169-174
Year: 2017

ATTENUATION OF CADMIUM-INDUCED HAEMATOLOGICAL DERANGEMENTS IN WISTAR ALBINO RATS BY *IRVINGIA GABONENSIS* O'RORKE BAILL ETHANOL LEAF EXTRACT

***Efosa Godwin Ewere[1], Oboso E. Etim[1], Samson Adewale Oyebadejo[2] and Blessing Victor Edem[1]**

[1]Biochemistry Department, Faculty of Basic Medical Sciences, University of Uyo P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.
[2]Anatomy Department, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria.

***Corresponding Author: Dr. Efosa Godwin Ewere**
Biochemistry Department, Faculty of Basic Medical Sciences, University of Uyo P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.

Article Received on 14/02/2017 **Article Revised on 05/03/2017** **Article Accepted on 25/03/2017**

ABSTRACT
Several health abnormalities such as derangement of haematological indices have been attributed to cadmium intoxication. The effect of *Irvingia gabonensis* O'Rorke Baill ethanol leaf extract on haematological indices in cadmium-exposed Wistar albino rats was investigated. 30 Wistar albino rats of weights between 88 and 148g were distributed into six groups of five animals each. Group 1 (normal control) received normal feeds and water *ad libitum*. Groups 2, 3 and 4 received 10mg/Kg body weight (mg/Kgbw) of cadmium chloride ($CdCl_2$), 10 mg/Kgbw $CdCl_2$ and 200 mg/Kgbw extract, 10 mg/kgbw $CdCl_2$ and 400 mg/Kgbw extract respectively. Groups 5 and 6 received 200 mg/Kgbw and 400 mg/Kgbw extract respectively for 28days. Treatment was by oral intubations. Results obtained showed significant ($p<0.05$) decreases in the levels of all assayed haematological indices when group 2 ($CdCl_2$ only) was compared with control with the exception of reticulocytes whose levels were significantly ($p<0.05$) increased, indicating anaemia. Treatment with the leaf extract significantly ($p<0.05$) attenuated the levels of all the haematological indices most of which were dose-dependent. The leaf extract also significantly ($p<0.05$) ameliorated perturbations in white blood cell differentials induced by cadmium when group 2 was compared with control apart from basophils whose levels were not significantly different. *Irvingia gabonensis* O'Rorke Baill ethanol leaf extract may be very useful in mitigating cadmium-induced haematological disturbances.

KEYWORDS: Anaemia, Cadmium, Haematological, Intoxication, *Irvingia gabonensis* O'Rorke Baill.

1.0. INTRODUCTION
Cadmium is a heavy metal and an environmental pollutant that poses a serious environmental hazard for human health.[1] It is naturally found in the environment as a constituent of ocean water and to a lesser level in surface water and groundwater. Cadmium is naturally emitted into the environment through volcanic activities, forest fires and generation of sea salt aerosols.[2] It can also be introduced in the environment through anthropogenic activities such as use of phosphate fertilizers, fossil fuel combustion and some industrial activities like welding and soldering.[3] Human exposure is primarily from fossil fuel combustion, natural sources, iron and steel production, cement production and related activities, nonferrous metals production and municipal solid waste incineration. Cadmium is toxic to human as excessive exposure can cause death.[4] It enters cells and accumulates in cytoplasmic and nuclear space in high concentrations.[5] Cadmium is highly a human carcinogen, which affects organs like the liver, kidneys and many bodily processes such as lipid metabolism, hematological functions amongst others.[1] It is a risk factor associated with a large number of illnesses including atherosclerosis, hypertension, cardiovascular disease and blood disorders.[3]

Medicinal plants have been employed over the years for preventive, therapeutic and curative purposes. *Irvingia gabonensis* O'Rorke Baill commonly called bush mango or African mango as the trees bear small mango-like fruits[6], is a tropical forest tree commonly found in Southern and Eastern Nigeria, Sierra Leone and Equatorial Africa and it belongs to the family, Irvingiaceae. The fruit pulp is sweet and edible with a characteristic turpentine flavor.[7] Traditionally, the leaves are widely used for the treatment of several illnesses.[8] The aqueous maceration of the leaves is used as antidote for some poisonous substances. The leaves are used to stop haemorrhage in pregnant women when combined with palm oil. The decoction of the stem bark

is employed in the treatment of gonorrhoea, hepatic and gastrointestinal disorders in Senegal.[9] The leaf extracts of the plant have been reported to have diuretic effect in rats and hypotensive effect in cats.[10], [11] The stem bark of the tree is added to palm wine as a preservative.[12] With the widely acclaimed medicinal properties of *Irvingia gabonensis* O'Rorke Baill leaf, this study was carried out to investigate the effect of *Irvingia gabonensis* O'Rorke Baill ethanol leaf extract on cadmium-induced haematological disturbances in Wistar albino rats.

2.0. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Cadmium chloride ($CdCl_2$) was purchased from Kermel chemical co. ltd, China. Absolute ethanol was purchased from JHD chemicals, China. All other reagents and chemicals used were of analytical grade.

2.2. Collection and preparation of plant sample

Fresh and matured leaves of *Irvingia gabonensis* O'Rorke Baill were harvested from Amanagwu village in Arochukwu Local Government Area of Abia State, Nigeria. The leaves were identified by Mr. Daniel Etefia of the department of Pharmacognosy and Herbal Medicine, Faculty of Pharmacy, University of Uyo, with the Herbarium no. James Daniel UUH 042116 (Uyo). The leaves were washed with distilled water and air-dried for seven days in Biochemistry laboratory, University of Uyo. The dried leaves were then pulverized using manual grinder and macerated in 80% ethanol for 72 hours while stirring at regular intervals for proper extraction. After 72 hours, the supernatant was carefully decanted and concentrated to dryness using a water bath at 45ºC. The concentrated extract was then stored in the freezer prior to use.

2.3. Experimental design

Thirty (30) Wistar albino rats of weights between 88–148g were obtained from the animal house, Derindam Research Institute of Biotechnology, Uyo, Akwa Ibom state, Nigeria. They were divided into six (6) groups of five (5) animals each in standard cages in a well ventilated room under standard conditions and allowed to acclimatize for 34 days. They were fed normal rat feed and water *ad libitum*. Treatment was done once daily by oral intubation and lasted for 28 days as shown in table 1 below.

Table 1: Protocol for Treatment of Experimental Animals

Experimental Groups	Treatment
Group 1 (control)	Normal feed and Water
Group 2	10mg/kgbw $CdCl_2$
Group 3	10mg/kgbw $CdCl_2$ + 200mg/kgbw ELEIG
Group 4	10mg/kgbw $CdCl_2$ + 400mg/kgbw ELEIG
Group 5	200mg/kgbw ELEIG
Group 6	400mg/kgbw ELEIG

ELEIG = Ethanolic Leaf Extract of *Irvingia gabonensis* (O' Rorke) Baill, bw = body weight.

2.4. Collection of blood samples

After the last treatment, the animals were fasted overnight with access to water only. They were anaesthetized using ketamine hydrochloride and blood was collected by cardiac puncture using sterile needles and syringes into labeled Ethylenediaminetetraacetic acid (EDTA) bottles and used for the haematological analyses.

2.5. Haematological analyses

White blood cell (WBC) count and its differentials were done using haematocytometer.[13] Red blood cell (RBC) count was done using haematocytometer.[14] Haemoglobin concentration was estimated using Cayman assay kits, U.S.A. according to manufacturer's protocol.

2.5.1. Determination of packed cell volume (PCV)

Wintrobe method was used. The wintrobe microhaematocrit tube was filled with blood by capillary action up to 2/3. The samples were spun for 5 minutes at 10,000rpm and the PCV was read as a percentage using the designed scale reader.

2.5.2. Platelet count

Blood was diluted with ammonium oxalate that haemolysed the RBC leaving Platelet intact and was counted using haemocytometer. 1:200 dilution (20µl: 3980µl) was made, the counting chamber was charged with well mixed diluted blood and the chamber was allowed to rest for 5minutes undisturbed. Platelets were counted using X40 objective in the large four corner area of the squares of the haemocytometer, number of cell count was calculated as

Platelet = N x 500 (500N).

2.5.3. Reticulocyte Count

Blood samples were kept in EDTA bottles; reticulocytes were demonstrated with supravital stain in which RNA appears as a network strand or granule. Equal volumes of blood and supravital stains were mixed in tubes, allowed to stand for 15 minutes, thin films were prepared and allowed to dry and were examined using X100 objective. 1000 RBCs that do not overlap were counted and reticulocytes were counted as well in each field and the following formula below was used for the estimation:

Retics = Retics counted x 100/RBC counted + Retics counted.

Mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV) were determined by calculations as follows:

$$MCHC = \frac{\text{Haemoglobin/100ml of blood}}{\text{Volume of packed cells/1000ml of blood}} = X100g/dl \text{ or } \%$$

$$MCH = \frac{\text{Haemoglobin/1000ml of blood}}{\text{Red blood cell count in millions/ml}} = Pg/cell$$

$$MCV = \frac{\text{Volume of packed cells/1000ml of blood}}{\text{Red blood cell count in millions/ml}} = flormm^3$$

2.6. Data analysis

Results were presented as mean ± standard deviation (SD) and were analysed using by one – way analysis of variance (ANOVA) for differences between groups with the aid of SPSS Software. P values less than (<) 0.05 were considered statistically significant for differences between means.

3.0. RESULTS

3.1. Effects *Irvingia gabonensis* ethanolic leaf extract on white blood cells and its differentials in experimental animals

As shown in table 2 below, there were significant decreases ($p<0.05$) in white blood cell count, monocytes, neutrophils and platelets count and significant increases ($p<0.05$) in lymphocytes and eosinophils in group treated with cadmium only (group 2) when compared with control. The lymphocytes and eosinophils of group 2 were significantly ($P<0.05$) higher when compared to all other groups. There was no significant ($P>0.05$) difference in basophils count across the groups.

Table 2: Effects *Irvingia gabonensis* ethanolic extract leaf on white blood cells and its differentials in experimental animals

Groups	WBC (µl)	LYM(µl)	MON (µl)	BAS (µl)	EOS(µl)	NEU(µl)	PLT (µl)
1	7.76±0.05^{bcde}	6.78±0.23^{bcde}	0.12±0.04^{bc}	0.02±0.04	0.11±0.02 ^{bcdef}	1.28±0.05 ^{bcdf}	918.40±71.35 ^{bcdef}
2	0.55±0.05^{acd}	8.53±0.45^{acd}	0.02±0.01^{ad}	0.05±0.01	0.19±0.03 ^{acd}	0.05±0.02 ^{acd}	337.00±55.55 ^{acd}
3	1.89±0.31^{abd}	1.89±0.31^{abd}	0.02±0.01^{ad}	0.02±0.01	0.02±0.01^{ab}	0.51±0.15^{ab}	459.80±65.56 ^{ab}
4	5.70±0.88^{abc}	3.41±1.21^{abc}	0.12±0.02^{bc}	0.01±0.01	0.02±0.01^{ab}	0.48±0.29^{ab}	506.00±133.59 ^{ab}
5	6.93±0.38^{a}	4.80±0.64^{af}	0.11±0.02^{f}	0.03±0.05	0.01±0.01^{a}	1.18±0.08^{f}	519.25±52.35 ^{af}
6	7.66±0.79	6.44±0.43^{e}	0.14±0.02^{e}	0.004±0.005	0.004±0.005^{a}	0.40±0.09^{ae}	649.60±29.18 ^{ae}

a= mean difference is significant ($P<0.05$) compared to group 1; b= mean difference is significant ($P<0.05$) compared to group 2; c= mean difference is significant ($P<0.05$) compared to group 3; d= mean difference is significant ($P<0.05$) compared to group 4; e= mean difference is significant ($P<0.05$) compared to group 5; f= mean difference is significant ($P<0.05$) compared to group 6; WBC= white blood cells; LYM=Lymphocytes; MON=Monocytes; EOS= Eosinophils; NEU=Neutrophils; PLT=Platelets

3.2. Effects *Irvingia gabonensis* ethanolic extract leaf on RBC, MCHC, MCH, MCV and PCV in experimental animals

The red blood cells, haemoglobin, MCHC, MCH, MCV and PCV of group 2 (cadmium only) were significantly reduced ($p<0.05$) when compared to all other groups. The reticulocytes of group 2 was significantly higher ($p<0.05$) when compared to groups 1, 3 and 4 as shown in table 2 below.

Table 3: Effects of *Irvingia gabonensis* O' Rorke Baill on RBC, MCHC, MCH, MCV and PCV in experimental animals

Groups	RBC (µl)	Hb (g/dl)	MCHC (g/dl)	MCH (pg)	MCV (fl)	PCV (%)	RET (l)
1	8.54±0.60^{bcde}	16.54±0.51^{bcdef}	33.80±2.39^{bc}	18.40±1.14^{bc}	54.00±1.58^{bcde}	49.40±1.34^{bcde}	120.2 ±24.50^{b}
2	3.83±0.82^{acd}	9.75±0.79^{acd}	20.28±1.62^{acd}	13.90±0.83^{acd}	34.31±5.12^{acd}	26.20±3.87^{acd}	295.25±137.69^{acd}
3	6.31±0.33^{abd}	12.78±0.98^{abd}	30.46±2.84^{ab}	16.48±0.64^{ab}	41.64±2.19^{ab}	34.60±2.97^{abd}	123.50±19.82^{b}
4	7.68±0.34 ^{abc}	14.30±0.85 ^{abc}	32.25±2.50 ^{b}	17.63±1.25 ^{b}	45.00±1.41^{ab}	38.75±3.69 ^{abc}	130.75±8.30^{b}
5	7.60±0.42 ^{a}	14.05±0.39 ^{af}	32.65±2.18	17.13±2.17	45.00±2.45^{af}	41.50±3.11^{af}	148.50±6.61
6	7.94±0.52	15.27±0.94 ^{ae}	35.20±1.30	18.66±1.06	52.20±2.77^{e}	49.20±2.59 ^{e}	196.00±54.57

a= mean difference is significant ($P<0.05$) compared to group 1; b= mean difference is significant ($P<0.05$) compared to group 2; c= mean difference is significant ($P<0.05$) compared to group 3; d= mean difference is significant ($P<0.05$) compared to group 4; e= mean difference is significant ($P<0.05$) compared to group 5; f= mean difference is significant ($P<0.05$) compared to group 6; Hb= Hemoglobin, MCHC = Mean Cell Haemoglobin Concentration, MCH = Mean Cell Haemoglobin, MCV = Mean Cell Volume, PCV = Packed Cell Volume, RBC = Red Blood Cell, RET= Reticulocytes.

4.0. DISCUSSION

The use of toxic chemicals, xenobiotics or certain synthetic compounds for various industrial and agricultural purposes has increased the pollution problems globally. Of these compounds, heavy metals induce potential effects even at low doses.[15] This has contributed to the increased use of medicinal plants and medicinal plant products, especially in Africa and North America, owing to their constituent secondary metabolites.[16]

In the present study, the effect of *Irvingia gabonensis* ethanol leaf extract on cadmium-induced haematological derangements was investigated. The number of white blood cells, monocytes, neutrophils and platelets in group 2 (cadmium only treated group) were significantly ($P<0.05$) decreased when compared with control, groups 3 and/or 4. This may be due to the oxidative stress induced by cadmium as oxidative stress is one of the mechanisms of cadmium toxicity via the generation of reactive oxygen species[17], hydrogen peroxide,[18] hydroxyl radicals[19] ultimately altering the antioxidant system in animals as a result of reduced glutathione levels and increased lipid peroxidation which is a primary mechanism for cadmium-induced toxicity.[20],[21],[22] The free radicals produced in this process are attached to any available molecule in intracellular environment culminating in cellular damage.[23],[24] This could culminate into impairment in immune function as white blood cells are majorly involved in immune protection thus exposing the system to attacks by foreign invaders.[25] The observed significant ($P<0.05$) decrease in platelets may inhibit the formation of platelet plugs needed to prevent haemorrhage at the site of injuries as well as loss of integrity of the capillaries.[26] This contradicts the findings of Hounkpatin *et al.*,[26] who reported significant increases with both low and high doses of cadmium in experimental rats compared with control. Furthermore, exposure to cadmium only in this study significantly ($P<0.05$) increased lymphocyte and eosinophils counts when group 2 (cadmium only) was compared with the control. Persistent changes are known to occur in the immune system during exposure to environmental pollutants[27] of which cadmium is one. There were no significant ($P>0.05$) differences in basophil counts across all experimental groups.

Treatment with *Irvingia gabonensis* ethanol leaf extract at different doses significantly ($P<0.05$) ameliorated the observed derangements in the aforementioned haematological parameters when the groups that were administered cadmium and extract simultaneously (groups 3 and 4) were compared with group 2 (cadmium only). The observed ameliorative effect of the leaf extract may owe to its very high antioxidant capacity[28] since one major mechanism of cadmium toxicity is by the induction of oxidative stress. Ojo *et al.*,[29] had reported the ameliorative effect of *Irvingia gabonensis* stem bark extract on cadmium-induced disturbances (in the aforementioned parameters) in wistar albino rats.

Red blood cells are majorly involved in the transport of dissolved oxygen due to their constituent oxygen-carrying pigment, haemoglobin. A decreased concentration of red blood cells and the oxygen-carrying haemoglobin is a sign of anaemia. In the present study, exposure of the experimental rats to cadmium only significantly ($P<0.05$) decreased red blood cell count, haemoglobin concentration, mean cell haemoglobin concentration (MCHC), mean cell hemoglobin (MCH), mean cell volume (MCV) and packed cell volume (PCV) when group 2 (cadmium only) was compared with the control. The observed significant decrease in red blood cell count may be due to the cytotoxic effect of cadmium as cadmium is known to cause oxidative stress via the production of reactive oxygen species (ROS) which could have compromised the red cell membrane integrity. Long-term cadmium exposure in rats has also been reported to culminate in anaemia due to the inhibition of the synthesis of erythropoietin, a hormone that stimulates the formation of red blood cell.[30] The decrease in haemoglobin concentration may be due to either an increase in the rate of haemoglobin destruction or to a decrease in the rate of haemoglobin synthesis.[31] These may have contributed to the observed significant decreases in PCV, MCHC, MCH and MCV in this study. The observed disturbances may be indicative of hypochromic and microcytic anaemia. This corroborates the findings of Ita and Udofia,[32] who reported the decreases in these parameters in Wistar rats exposed to crude petroleum, petrol, kerosene and diesel. The reticulocyte count in this study showed a significant increase ($p<0.05$) in cadmium exposed group when compared with control, groups 3 and 4. This increase could be attributed to the increased need for red blood cell formation in the bone marrow as red cell haemolysis stimulates the production of reticulocytes.[25]

Treatment with the ethanol leaf extract of *Irvingia gabonensis* O'Rorke baill at different doses significantly ($P<0.05$) increased the RBC, MCHC, MCH, MCV, PCV and significantly decreased the reticulocytes counts when the extract treated groups were compared with the cadmium exclusively exposed group. This may as well point to the very high antioxidant power of the leaf extract[28] thus mitigating the effect of cadmium-induced ROS generation thereby inhibiting the destruction of red cell membrane. The protective effect of the leaf extract may also not be unconnected to its very high iron content.[33] Iron helps in the synthesis of haemoglobin and thus transportation of oxygen. It also aids the synthesis and packaging of neurotransmitters, their uptake and degradation into other iron-containing proteins which may directly or indirectly alter brain function.[34] Onwuka *et al.*,[35] also reported a significant decrease in the haemoglobin concentration in cadmium exposed rats when compared to the control; treatment

with cabbage supplement however significantly increased the haemoglobin concentration.

5.0. CONCLUSION

The present study has revealed the ameliorative ability of ethanol leaf extract of *Irvingia gabonensis* O'Rorke baill on cadmium-induced haematological disturbances in Wistar albino rats. Ethanol leaf extract of *Irvingia gabonensis* O'Rorke baill may therefore be very useful in mitigating haematological disturbances induced by cadmium.

REFERENCES

1. Godt J, Scheidig F, Grosse-Siestrup C, Esche V, Brandenburg P, Reich A, Groneberg DA. The toxicity of cadmium and resulting hazards for human health. *J Occup Med Toxicol*. 2006; 1: 22.
2. ATSDR. Toxicological profile for cadmium. Agency for Toxic Substances and Disease Registry. ATSDR/U.S. Public Health Service, 1989; Atlanta/TP-88/08.
3. Morrow, H. Cadmium and Cadmium Alloys. Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley & Sons. 2010; 1–36.
4. Othumpangat S, Kashon M and Joseph P. Eukaryotic translation Initiation Factor 4E is a cellular target for toxicity and death due to exposure to cadmium chloride. *J. Biol Chem*., 2005; 280: 162-69.
5. Beyersmann D and Hechtenberg S. Cadmium, gene regulation and cellular signaling in mammalian cells. *Toxicol. Appl. Pharmacol*., 1997; 144: 247-61.
6. Matos L, Nzikou JM, Matouba E, Pandzou-Yembe VN, Mapepoulou TG, Linder M and Desobry S. Studies of Irvingia gabonensis seeds kernels: Oil technological applications. *Pakistan Journal of Nutrition*, 2009; 8: 151-57.
7. Udeala O, Onyechi J. and Agu S. Sourcing Pharmaceutical Raw Materials from Indigenous Medicinal plants. *Journal of Pharmacy and Pharmacology*, 1980; 32: 36.
8. Lowe AJ, Gillies ACM, Wilson J. and Dawson IK. Conser-vation genetics of Bush Mango from Central/West Africa: Implica-tions for RAPD analysis. *Molecular Ecology*, 2000; 9(7): 831 – 41.
9. Hubert DJ, Wabo FG, Ngameni B, Ngheguin TF, Tchoukoua A, Ambassa P, Abia W, Tchana AN, Giardina S, Buonocore D, Vita FP, Vidari G, Marzatico F, Ngadjui BT. and Moundipa PF. "In vitrohepatoprotective and antioxidant activities of the crude ex-tract and isolated compounds from Irvingia gabonensis. Asian *Journal of Traditional Medicine*, 2010; 5(3): 79-88.
10. Nosiri, C, Abdu-Aguye, I, Hussaini, I and Abdurahaman, E. Leaf Extracts of *Irvingia gabonensis* Increase Urine Output and Electrolytes in Rats. *The Internet Journal of Alternative Medicine*, 2009a; 8(2): http://ispub.com/IJAM/8/2/11789.
11. Nosiri, C., Hussaini, I., Abdu-Aguye, I. and Abdurahaman, E. Pharmacological Effect of *Irvingia gabonensis* Leaf Extracts on Cat Blood Pressure. *The Internet Journal of Pharmacology*, 2009b; 9(1): http://ispub.com/IJPHARM/9/1/6633.
12. NAERLS (National Agricultural Extension and Research Liaison Services). Production and utilization of Ogbono (*Irvingia gabonensis*). Extension bulletin No 140. Horticulture series No 4. 1999; 3-12.
13. Cheesbrough, M. District laboratory practice in tropical countries. Part 1 & 2. Cambridge University Press, United Kingdom, 2004; 208-55.
14. Ochei J and Kolhatkar A. Medical laboratory sciences: Theory and practice. Tata McGraw Hill, New York, 2008; 663-65.
15. Jagadeessan, G and Pillai, SS. Hepatoprotective Effect of Taurine against Mercury Induced Toxicity in Rat. *Journal of Enviromental Biology*, 2007; 28: 753-56.
16. Briskin, DP. Medicinal Plants and Phytomedicine. Linking Plant Biochemistry and Physiology to Human Health. *Plant Physiology*, 2000; 507 – 14.
17. Amoruso, MA. Witz, G and Goldstein, BD. Enhancement of rat and human phagocyte superoxide anion radical production by cadmium *in vitro*. *Toxicology Letters*, 1982; 10: 133-8.
18. Wong, Z, Troll, W, Koenig, KL, Frenkel, K. Carcinogenic sulfide salts of nickel and cadmium-induced H_2O_2 formation by human polymorphonuclear leukocytes. *Cancer Research*, 1990; 20: 7564-70.
19. Ochi, T, Otsuka, F, Takahashi, K and Oshawa, M. Glutathione and metallothioneins as cellular defense against cadmium toxicity in culture Chinese hamster cells. *Chemico-biology interactions*; 1998; 65: 1-14.
20. Zikic, RV, Stajn, A, Saicic, ZS, Spasic, MB, Ziemnicki, K and Petrovic, VM. The activities of superoxide dismutase, catalase and ascorbic acid content in the liver of goldfish (Carassius au-ratus gibelio Bloch.) exposed to cadmium. *Physiology Research* 1996; 4596: 479-81.
21. Bagchi, D, Vuchetich, P, Bagchi, M, Hassoun, EA, Tran, MX, Tnag, L and Stohs, SJ. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free Radical Biology and Medicine*, 1997; 22: 471-8.
22. Eneman, JD, Potts, RJ, Osier, M., Shukla, GS, Lee, CH.Chin, JF and Hart, BA. Suppressed oxidant induced apoptosis in cadmium adapted alveolar epithelial cells and its potential involve-ment in cadmium carcinogenesis. *Toxicology*, 2000; 7: 215-28.
23. Yiin, SJ, Chern, CL, Sheu, JY, Tseng, WC, and Lin, TH. Cadmium-induced renal lipid peroxidation in rats and protection by selenium. *Journal of Toxicology and Environmental Health*, 1999; 57(6): 403-13.

24. Murugavel, P and Pari, L. Effect of diallyl tetrasulfide on cad-mium-induced oxidative damage in the liver of rats. *Human and experimental toxicology*, 2007; 26(6): 527-34.
25. Udombon, NS. Textbook of Practical Physiology, 2nd edition, Jiano Media. 2013; 77 – 101.
26. Hounkpatin, ASY, Johnson, RC, Guédénon, P, Domingo, E, Alimba, CG, Boko, M and Edorh, PA. Protective Effects of Vitamin C on Haematological Parameters in Intoxicated Wistar Rats with Cadmium, Mercury and Combined Cadmium and Mercury. *International Research Journal of Biological Sciences*, 2012; 1(8): 76-81.
27. Devereux, G, Barker, RN and Seaton, A. Antenatal Determinants of Neonatal Immune Reponses to Allergens. Clinical and Experimental Allergy. *Journal of the British Society for Allergy and Clinical Immunology*, 2002; 32: 43 – 50.
28. Ewere, EG, Uka, E, and Usunobun, U. Phytochemical Composition, Invitro Antioxidant Activity and Acute Toxicity of *Irvingia gabonensis* (O'Rorke) Baill Ethanolic Leaf Extract. *International Journal of Biological Research*, 2016; 4(1): 36-41
29. Ojo, OA, Ajiboye, BO, Oyinloye, BE and Ojo, AB. He-matological Properties of *Irvingia gabonensis* in Male Adult Rats. *Journal of Pharmaceutical and Scientific Innovation*, 2014; 3(5): 434-36.
30. Horiguchi, H, Sato, M, Konno, N, and Fukishima, M. Long-term Cadmium Exposure Induces Anemia in Rats through Hypo Production of Erythropoietin in the Kidneys. *Arch Toxicol.*, 1996; 71: 11-19.
31. Reddy, PM, and Bashamohideen, M. Fenvalerate and Cypermethrin Induced Changes in the Haematological Parameters of *Cyprinu carpio*. *Acta Hydrochem Hydrobiology*, 1989; 17: 101-07.
32. Ita SO and Udofia UA. Comparative Study of Some Haematological Parameters in Rats Following Ingestion of Crude Oil (Nigerian Bonny Light), Petrol, Kerosene and Diesel. *Asian Journal of Biological Sciences*, 2011; *4: 498-505*.
33. Ewere, EG, Etim, OE and Usunobun, U. Proximate composition, mineral content and amino acid profile of *Irvingia gabonensis* O'Rorke baill leaf. *International Journal of Scientific World*, 2017; 5(1): 23-27.
34. Beard, JL. Iron biology in immune function, muscle metab-olism and neuronal functioning, *Journal of Nutrition.* 2001; 131: 5685-95.
35. Onwuka, FC, Erhabor, O, Eteng, MU and Umoh, IB. Ameliorative Effect of Cabbage Extract on Cadmium-induced Changes on Haematologyand Biochemical Parameters of Albino Rats. *Journal of Toxicology and Environmental Health Sciences*, 2010; 2(2): 11-16.