European Journal of Applied Sciences – Vol. 9, No. 5
Publication Date: October 25, 2021
DOI:10.14738/aivp.95.10963.
Olua, V., Nwaichi, E. O., & Wegwu, M. O. (2021). Toxicological Evaluation of Telfaria Occidentalis and Talinum Triangulare Grown in Freshly Recovered Petroleum-Polluted Soil. European Journal of Applied Sciences, 9(5). 262-271.

Toxicological Evaluation of Telfaria Occidentalis and Talinum Triangulare Grown in Freshly Recovered Petroleum-Polluted Soil

Olua, V.
World Bank Africa Centre of Excellence Centre for Oil Field Chemical Research (ACE-CEFOR) University of Port Harcourt, Nigeria

Nwaichi, E. O.
Department of Biochemistry, University of Port Harcourt, Nigeria

Wegwu, M. O.
Department of Biochemistry, University of Port Harcourt, Nigeria

ABSTRACT

Toxicity effect of *Telfaria occidentalis* and *Talinum triangulare* harvested from oil-polluted soil remediated using agro-waste formulations, on haematological indices of Wistar rats, was evaluated. Thirty-five rats grouped into seven of five rats each was used for this study. Group I received feed and water only, groups II & III received 1000mgkg^{-1} of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted soil respectively, groups V & VI received 1000mgkg^{-1} of *Telfaria occidentalis* and *Talinum triangulare* from polluted but amended soil respectively, while groups IV & VII received 500mgkg^{-1} each of *Telfaria occidentalis* and *Talinum triangulare* from unpolluted and polluted but amended soil respectively. The results ranged as follows; PCV (47.30±1.21 to 54.06±0.78), Hb (15.19±0.27 to 17.20±0.20), RBC (4.79±0.07 to 5.67±0.07), WBC (3.37±0.16 to 3.84±0.09), and Platelet count (157.50±0.07 to 193.17±0.20). Obtained results showed that PCV, Hb and RBC counts of wistar rats treated combined leaf extract of *Telfaria occidentalis* and *Talinum triangulare* harvested from the amended soil were significantly increased (@p≤0.05) compared with the control group. This however suggests that the aqueous extract of *T. occidentalis* and *T. triangulare* harvested from the crude oil impacted farm remediated using selected agro waste formulation had no probable acute toxicity effects on the haematological parameters of Wistar albino rats as obtained values were within the reference ranges. Levels of haematological parameters obtained indicates that the plant extracts still maintained their blood boosting potentials with improved ability in enhancing erythropoiesis.

Keywords: Toxicity effect, Bioremediation, haematological indices, Crude oil pollution, Agro-waste.

INTRODUCTION

A problem the global environment is suffering from today is the resultant effect of environmental contamination and pollution by natural disaster, equipment failure of industrial gadgets, oil leakages and spillages, management of waste materials, etc. Lots of approaches have been taken to remedy the situation [1,2]. Such approaches have focused on the use of microorganisms [3,4,5], while some deployed the use of plants and agrowastes [6]. Despite the

amendment methods, reports have shown that no hundred percent clean up level have been achieved from any polluted site [1,2,6]. This further entails some amount of the pollutants still remains within the contaminated soil but at levels assumed to be safe for life within the surrounding environment. This study however seeks to evaluate the effects of aqueous leaf extracts of *Telfaria occidentalis* and *Tallinum triangulare* harvested from bioremediated crude oil polluted soil on hematological profile of wistar rats.

Telfaria occidentalis and *Tallinum triangulare* (pumpkin leaves and water leaves) are commonly cultivated, harvested and sold in all markets within the Niger Delta region of Nigeria. They are consumed by over 60% of all households daily. Some of these vegetables were harvested from recovered sites, without recourse to contamination or pollution level. *Telfaria occidentalis* and *Talinum triangulare* in this study was planted and harvested on a crude oil polluted site remediated using some agro-wastes (rice husk, egg shell and plantain peels) formulations. This was done to ascertain potential toxicity effect of vegetables from such remediated sites (remediated using afore mentioned agro-waste formulations) on haematological indices of wistar rats.

MATERIALS AND METHODS

Plant sample collection and Preparation

Fresh waterleaf (*Talinum triangulare*) stems and pumpkin (*Telfaria occidentalis*) Seedlings were obtained from a Farm in Anambra Imo River Basin Development authority, Owerri in Imo State. The *Talinum triangulare* stems and *Telfaria occidentalis* Seedlings were planted on normal (unpolluted) control soil (obtained from the Faculty of Agriculture botanical farm, university of Port Harcourt) and crude oil polluted soil remediated using agro waste formulations. The leaves were harvested, air dried, ground into powder in preparation for aqueous extraction. The rotary extraction method was used for aqueous extraction of *Talinum triangulare* and *Telfaria occidentalis*.

Experimental Design

Healthy thirty five wistar rats of 9-12 weeks old of about 120-170g body weight grouped into seven with five rats each was used for acute toxicity study. They were purchased from the animal house of the Faculty of Basic medical sciences and housed in the Animal house of the Department of Biochemistry all in the University of Port Harcourt, Port Harcourt Rivers State Nigeria. The rats were maintained on growers mash (Top Feed Ltd.) and water *ad libitum* for seven (7) days to allow for acclimatization before commencement of various administrations.

URL: http://dx.doi.org/10.14738/aivp.95.10963

Table 1 Groupings of the Experimental Animals

Groups	Code	Treatment
I	Normal Control	The animals in this group were given distilled water and normal feed throughout the experiment.
II	NP	The animals received 1000mgkg^{-1} of *T. occidentalis,* from unpolluted soil water and normal feed
III	NW	The animals received 1000mgkg^{-1} of *T. triangulare,* from unpolluted soil, water and normal feed
IV	NP/NW	The animals received 500 mgkg^{-1} each of *T. triangulare* and *T. occidentalis,* from unpolluted soil, water and normal feed
V	TP	The animals received 1000mgkg^{-1} of *T. occidentalis,* from the remediated soil (bioremediated using agrowaste formulations), water and normal feed
VI	TW	The animals received 1000mgkg^{-1} of *Talinum triangulare,* from remediated soil (bioremediated using agrowaste formulations) , water and normal feed
VII	TP/TW	The animals received 500mgkg^{-1} of *Talinum triangulare* and *T. occidentalis* each*,* from remediated soil (bioremediated using agrowaste formulations), water and normal feed

On conclusion of the experiment, the Wistar rats were harvested after anaesthesising the wistar rats and sacrificing the Wistar rats via jugular puncture. Blood samples were taken to the laboratory for heamatological analysis.

Evaluation of Haematological Parameters and Indices

Haematological parameters (Hb, White Blood Cell count, Red Blood Cell count, Platelet count, Neutrophils, Lymphocytes, Monocytec (MONO), Eosinophils (EOS), Basophils (BAS) counts) and indices were assayed using Vet - Automated Haematology analyzer (Abacus junior, 1 Radim, Italy) 43. The automatic method (automatic cell counter) vet haematology analyzer was used (Abacus junior Radiun, Italy) after putting the samples on electric mixer. Each sample was estimated in triplicates.

Data/Statistical Analysis

Statistical analysis was done using SPSS Version 21.0 to determine the one way analysis of variance(Anova), and a descriptive multiple comparism of mean values (n=3) @ $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The results of the Effects of *T. triangulare* and *T. occidentalis* aqueous leaf extracts on the hematological parameters of Wistar Albino Rats is as shown in Table 2. The result revealed maximum PCV values (54.06 ±0.78%) in groups administered with combined leaf extract of *Talinum triangulare* (TW) and *T. occidentalis* (TU) TW/TU) obtained from soil regime treated with Agro-waste formulations that best remediated the sampled polluted soils, while minimum value (47.30±1.21%) was obtained in the group treated with waterleaf from polluted soil regime remediated with the best performance agrowaste formulation. The values obtained were shown to be significantly different from the control group (fed with feed and water only).

This findings corroborates earlier studies by Obeagu *et al.,* Toma *et al.,* Bashir *et al.,* and Etim *et al.,* who reported increased PCV in wistar rats fed with extracts from *T. occidentalis* [7,8,9,10]. The PCV results also corroborates the findings by Burns and De Lannoy and Jiwuba *et al.,* who reported PCV range indicating no toxicity effect of plant extracts (*M. Oleifera*) on experimental animal[11,12]. The findings however, gave results which are significantly different from the control (@ $p \leq 0.05$) but within recommended reference range [12], This shows that the sampled plant extracts could have boosting effects on PCV which is typically about 50-52% for men and 36-48% for women.

Results from the TW (group VI) though minimal was however seen to be within standard value, thereby depicting no probable diseased condition.

The Hb (17.20±0.07 g/dL) and RBC (5.670±0.07 cells/mcL) maximum values were seen in group administered with combined leaf extract of *Talinum triangulare* (TW) and *T. occidentalis* (TP) (TP/TW) obtained from soil regime treated with the best performance Agrowaste formulation, while minimum Hb (15.19±0.27 g/dL) seen in NP (pumpkin leaf obtained from normal unpolluted soil) treated group and RBC minimum value (4.79±0.08 cells/mcL) seen in control group. This study is suggestive that the *T. triangulare* and *T. occidentalis* leaf extracts effectively boosted HB and RBC levels in all extract administered groups (but more significantly is that from plant extracts from the best remediation formulation) thereby aiding in maintaining Hb and RBC level within the standard level. This findings are in consonance with reports by Obeagu *et al.,* and Etim *et al.,* [7,10]. The more significant effect seen in the plant extracts harvested from the bioremediated soil could also imply that the bioremediation formulation could have given a boost to the soil nutrient profile, hence furnishing the plants with required nutrients to thrive and also boost the afore mentioned hematological indices.

Decreased hematocrit denotes anemia (Aplastic anemia and thalassemia syndromes specifically). This can result from reduced number of erythrocytes, hemoglobin in each red blood cells or both. This often occur from damage to myeloid tissue by toxicants, (such as, heavy metals, benzene, polycyclic aromatic hydrocarbons) medications (e.g antibiotics), or radiation; which inhibit hematopoietic enzymes [13,14].

Studies have led to the proposition of Anemia diagnosis if a blood test finds less than 13.5 g/dL in a man or less than 12 g/dL in a woman's Hb levels and a varying levels in children. However this study have shown no toxicity effect from plants harvested from this bioremediated soil.

URL: http://dx.doi.org/10.14738/aivp.95.10963

Table 2 Heamatological Indices of Wistar Rats Fed with Plant Extracts

GROUP	PCV	Hb	WBC	RBC	PLT	NEU	LYMP	MONO	EOS	BAS
CONTROL	47.93±0.30[a]	15.54±0.18[a]	3.48±0.08[a]	4.79±0.07[a]	162.29±0.55[a]	19.19±0.26[a]	73.32±1.67[a]	4.67±0.38[a]	2.91±0.47[a]	0.19±0.02[a]
NP	51.46±0.58[b]	15.49±0.14[a]	3.52±0.00[a]	4.94±0.10[a]	157.50±0.07[a]	20.72±0.08[a]	73.66±01.0[a]	3.72±0.29[a]	1.17±0.09[a]	0.19±0.01[a]
NW	49.03±0.83[a]	15.19±0.27[a]	3.73±0.08[a]	5.22±0.06[a]	167.02±0.21[a]	23.97±0.47[a]	71.87±1.33[a]	4.25±0.27[a]	1.51±0.02[a]	0.45±0.10[a]
NP/NW	52.51±0.60[b]	16.20±0.03[b]	3.37±0.16[a]	5.10±0.08[a]	180.23±0.49[b]	18.81±0.16[a]	76.17±0.49[b]	3.60±0.34[a]	1.61±0.12[a]	0.16±0.03[a]
TP	51.81±0.81[b]	15.94±0.09[a]	3.61±0.15[a]	5.57±0.14[b]	165.18±0.01[a]	21.09±0.61[a]	72.92±1.08[a]	4.68±0.31[a]	1.66±0.07[a]	0.37±0.05[a]
TW	47.30±1.21[a]	15.53±0.18[a]	3.84±0.03[a]	4.97±0.09[a]	163.17±0.20[a]	23.70±0.30[a]	70.57±0.25[a]	3.76±0.37[a]	1.06±0.03[a]	0.11±0.01[a]
TP/TW	54.06±0.78[b]	17.20±0.07[b]	3.84±0.09[a]	5.67±0.07[b]	162.91±0.27[a]	24.63±0.33[a]	68.06±0.10[a]	4.39±0.10[a]	1.69±0.10[a]	0.34±0.12[a]

Values represents Mean±SEM. Mean in the same column with same superscript alphabets are not significantly different. NP = *T. occidentalis* planted on Amended Polluted soil, TW = *Talinum triangulare* planted on Amended Polluted soil, NP = *T. occidentalis* planted on Unpolluted control soil, NW = *Talinum triangulare* planted on Unpolluted control soil

Photomicrographs of the Blood, Magnification X100

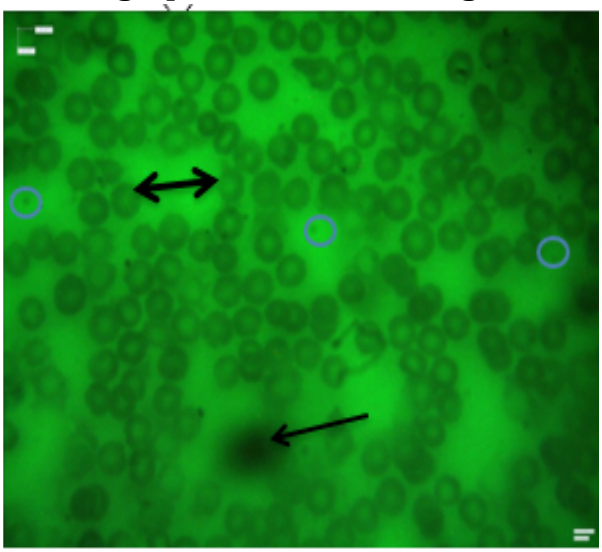


Plate 1. Control Group. The histomorphology of a normal blood smear stained with Giemsa stain at x100 magnification showed the various cell component of the blood. The platelet were seen circled as tiny dots in the field area while the numerous cells observed were Red Blood cells. These were pointed by a double edged arrow. The red blood cells are oval in shape and no observable ruptured red blood cells are seen in this field. The white lymphocyte seen in this field was point out using a single headed arrow. In general, necrotic cells are not present in this field.

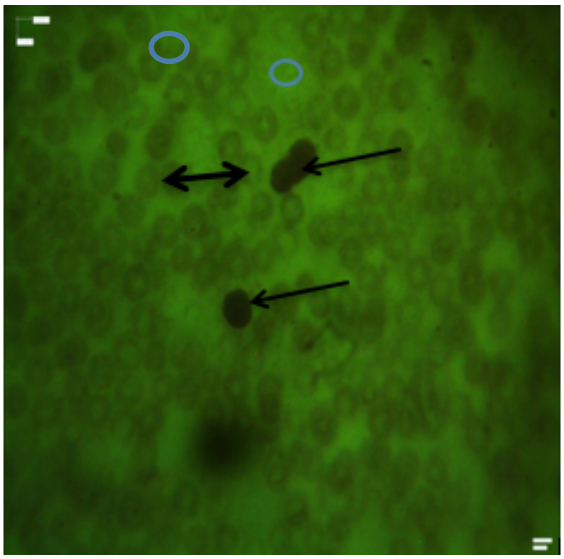


Plate 2. Group NP. More lymphocytes were observed in this group. The oval shapes of the red blood cells were slightly but not significantly distorted

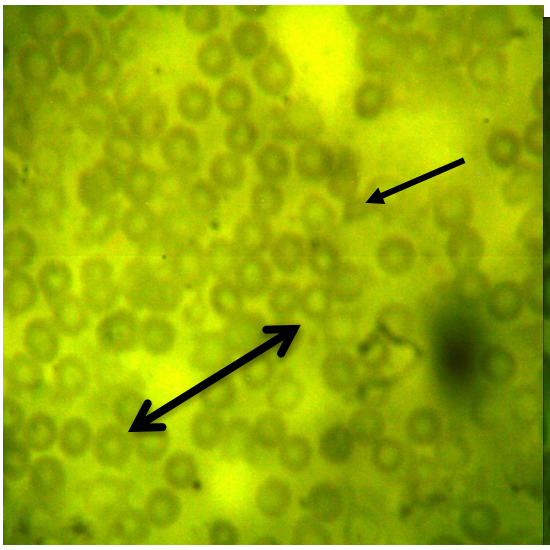


Plate 3. Group NW. An eosinophil was found just at the edge of the field. numerous cells observed were Red Blood cells. These were pointed by a double edged arrow. Few Lysed Red blood cells were also spotted.

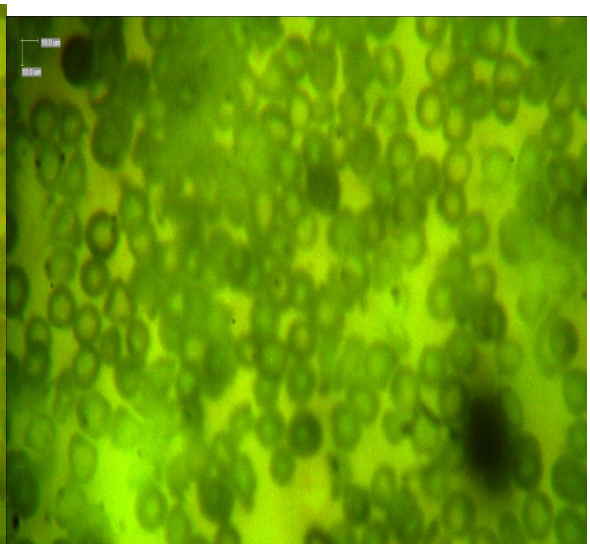


Plate 4. Group NP/NW. Majority of the cells in this field were Ruptured with few still maintaining their oval shape. Few lymphocytes can be sited in this field.

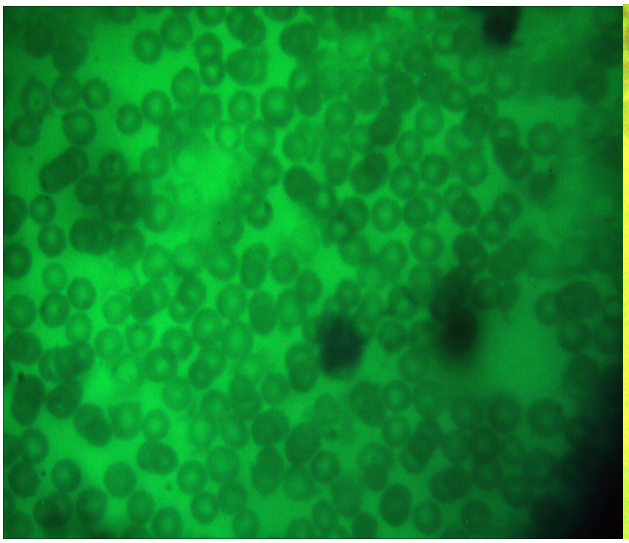


Plate 5. Group TP Red blood cells were observed with non significantly lysed and distorted cells all around the field. RBC were also seen maintaining their oval shape.

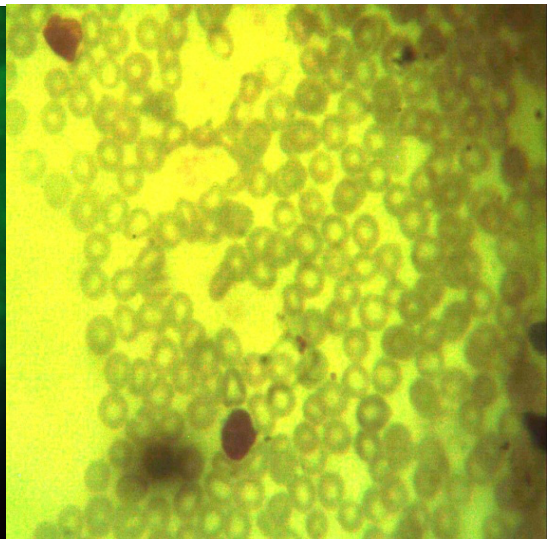


Plate 6. group TW. The RBC were densely populated

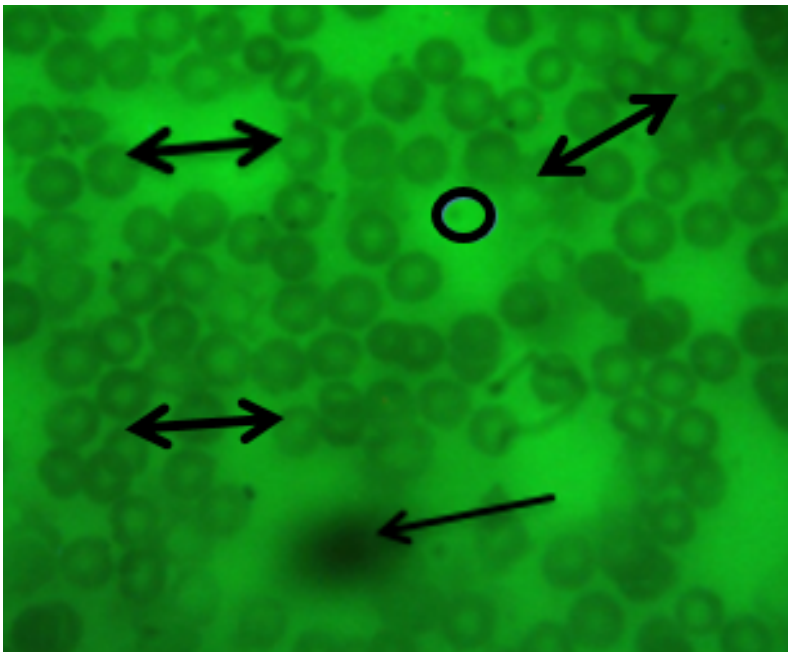


Plate 7 Group TP/TW. Few lymphocytes were observed and pointed out using single arrow. Tiny platelet droplets were circled. Numerous Red Blood cells were observed. These were pointed by double edged arrows. The red blood cells were oval in shape and no observable ruptured red blood cells were seen. In general, necrotic cells are not present in this field.

The WBC has minimum value (3.37±0.16) in NP/NW fed group with maximum value obtained from NP Group (3.840±0.09). The obtained results shows increased level of WBC, however values obtained were shown not to be significantly different from the control, same trend was also seen in the percentage counts for Neutrophils, Monocytes and Basophils. Eosinophil content was shown to have decrease across all extract treated groups when compared to control, though at levels seen to be statistically not significantly different. Lymphocytes also gave values which were not significantly different from the control group except for the groups treated with combined extracts of the leaves obtained from unpolluted soil. Same was seen for Platelets counts. The non significant values seen for WBCs in this study corroborates earlier reports by Adias *et al*., and Obagu *et al*., [7,15]. The significant increase in the PCV, Hb and RBC counts could be linked to Fe and Vit B complex contents of the sampled leaves [16]. White blood cell (WBC) count measures the number of white blood cells in the body. Having a higher or lower number of WBCs than normal may indicate an underlying disease condition which hence demands the need for proper mechanism to be deployed in restoring or maintaining WBC levels within the standard range (reference range for adults (males and females) is as follows: Total leukocytes: 4.00-11.0 x 10^9/L [17,18]. Differential WBC count is in percentage of overall WBC count neutrophil (55 to 73 percent), lymphocyte (20 to 40 percent), eosinophil (1 to 4 percent), Monocyte (2 to 8 percent), basophil (0.5 to 1 percent) [17,18]. The findings from this study shows that the treated groups gave WBC levels within the normal range. Obtained results however showed that *Talinum triangulare* (TW) and *T. occidentalis* (TP) aqueous leaf extracts obtained from the amended site had significant impact on hematological indices of Wistar albino rats compared to the control. This suggests that the extract could have great ability to stimulate erythropoietin formation and secretion leading to increased erythropoesis and hence, presents them useful as blood boosters. This is also an indication that *T. triangulare* and

T. occidentalis harvested from this remediated site are non-toxic in nature to red blood cells and general health status of the experimental animal at 1000mgkg^{-1} body weight dosage within administration period. The analysis of blood parameters is a means of establishing the state of health of an animal and thus determining the effect of the ingestion of food components on the blood composition [19]. Reports shows that hematological indices are useful in monitoring food toxicity [20] as changes in hematological parameters in animals is indicative of their health status.

The findings in this study is also evidenced in the photomicrograph of blood smears from the experimental animals. The Control Group given feed and water only as shown in Plate 1 showed various cell component of blood with numerous Red Blood cells, few platelets devoid of necrotic cells. No observable ruptured red blood cells were seen in this field. However few lymphocytes were seen. On Plate 2 treated with *T. occidentalis* from unpolluted soil the photomicrograph showed more lymphocytes with red blood cells slightly but not significantly distorted. While the group treated with *T. triangulare* from unpolluted soil as shown in plate 3 revealed presence of sparsely placed eosinophil with few lysed Red blood cells also spotted. Plate 4 shows blood photomicrograph of the group treated with combined extract of *T. triangulare* and *T. occidentalis* from unpolluted soil. It revealed fields with few Ruptured RBC and few lymphocytes sited. In the group treated with *T. occidentalis* from polluted soil but amended with formulated agro wastes (Plate 5), Red blood cells were observed with non significantly lysed and distorted cells all around the field. RBC were also seen maintaining their normal shape. Plate 6. (group treated with *T. triangulare* from polluted soil but amended with formulated agro wastes), showed densely populated RBC. While Plate 7 (Group treated with combined extract from *T. triangulare* and *T. occidentalis* from polluted soil but amended with formulated agro wastes), showed presence of Few lymphocytes, Tiny platelet droplets, numerous Red Blood cells with no observable rupture nor necrotic cells.

CONCLUSION

This study is suggestive that the aqueous extract of *T. occidentalis* and *T. triangulare* harvested from the crude oil impacted farm remediated using selected agro waste formulation had no probable acute toxicity effects on the heamatological (blood) parameters of sampled wistar albino rats. This however, is indicative that harvested plants from this remediated soil are devoid of toxicity effects making the agro-waste formulation (used for the bioremediation process) potentially fit for amendment of crude oil impacted site to levels that harvested plants, planted immediately after remediation duration could be devoid of pollutants capable of inducing acute toxicity effects on exposure to such harvested plants. Levels of hematological parameters obtained indicates that the plant extracts still maintained their blood boosting potentials with improved ability in enhancing hematological indices as compared to the control.

URL: http://dx.doi.org/10.14738/aivp.95.10963

References

[1]. Dados, A., Omirou, M., Demetriou, K., Papastephanou, C. and Ioannides, I.M. (2015). Rapid remediation of soil heavily contaminated with hydrocarbons: a comparison of different approaches. *Annuals of Microbiology* **65**:241–251.

[2]. Azubuike, C.C., Chikere, C.B. and Okpokwasili, G.C. (2016). Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World Journal Microbiology Biotechnology* **32**:1–18.

[3]. Adebusoye, S.A., Ilori, M.O., Amund, O.O., Teniola, O.D. and Olatope, S.O. (2007). Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World Journal of Microbiology and Biotechnology* **23**:1149–1159.

[4]. Silva-Castro, G.A., Uad, I., Rodrı´guez-Calvo, A., Gonza´lez- Lo´pez, J. and Calvo, C. (2015). Response of autochthonous microbiota of diesel polluted soils to land-farming treatments. *Environmental Res*earch. **137**:49–58.

[5]. Chioma, B. C., Christopher, C. A. and Evan, M. F. (2017). Shift in microbial group during remediation by enhanced natural attenuation (RENA) of a crude oil-impacted soil: a case study of Ikarama Community, Bayelsa, Nigeria. *Biotechnology*, **7**:152-163

[6]. Nwaichi, E. O., Magdalena, F., Paul, A. N. and Progress, E. (2015) Enhanced Phytoremediation of Crude Oil-Polluted Soil by Four Plant Species: Effect of Inorganic and Organic Bioaugumentation, *International Journal of Phytoremediation*, **17**:(12), 1253-1261

[7]. Obeagu, E.I., Chikelu, I.M., Obarezi, T.N., Ogbuabor, B.N. and Anaebo, Q.B. (2014). Haematological effects of Fluted Pumpkin (Telfairia Occidentalis) Leaves in Rats. *Int J Life Sci Biotechnol Pharma Res.* 3(1):1-11.

[8]. Toma, I., Victory, N.C. and Kabir, Y. (2015). The effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine- induced anemic rats. *Afr J Biochem Res.* 9(7):95-98.

[9]. Bashir, L., Oluwatosin, K.S., Agboola, A.R., Ibrahim, A.O., Adeniyi, A.K. and Prince, C.O. (2015). Effect of Methanol Extract of Telfairia occcidentalis on Haematological Parameters in Wister Rats. *J Med Sci* 2015;15(5):246-250.

[10]. Etim, O. E., Akpan, H. D. and Usuah, J. A. (2015). Effects of Co-Administration of Aqueous Extracts of Telferia occidentalis and Ageratum conizoides On Haematological Indices, Urea and Creatinine in Albino Wistar Rats. *Eur J Biol Med Sci Res* ;3(4):1-10.

[11]. Burns, K.F. and De Lannoy, C.W. (1966). Compendium of normal blood values of laboratory animals, with indication of variations: I. Random-sexed populations of small animals. *Toxicology and Applied Pharmacology*. **8**(3):429-437

[12]. Jiwuba, P., Ikwunze, K., Dauda, E. and Ugwu, D. (2016). Haematological and serum biochemical indices of growing rabbits fed diets containing varying levels of *Moringa oleifera* leaf meal. *British Biotechnology Journal* 15(2):1-7.

[13]. Tamariz, L.J., Young, J.H., Pankow, J.S., Yeh, H.C. and Schmidt, M.I. (2008). Blood viscosity and hematocrit as risk factors for type 2 diabetes mellitus: the atherosclerosis risk in communities (ARIC) study. *Amercan Journal of Epidemiology.* 168: 1153-1160.

[14]. Oduola, T., Adeniyi, F., Ogunyemi, E., Bello, I., Idowu, T. and Subair, H. (2007). Toxicity studies on an unripe Carica papaya aqueous extract: biochemical and haematological effects in wistar albino rats. *Journal of Medicinal Plants Research* 1(1):001-004.

[15]. Adias, T.C., Ajugwo, A.O., Erhabor, T., Nyenke, C.U.. (2013). Effect of Pumpkin Extract (Telfairia occidentalis) on Routine Haematological Parameters in Acetone-Induced Oxidative Stress Albino Rats. *American Journal of Food Science Technology*. 1(4):67-69.

[16]. Ganong, W.F. (2005). A review of medical physiology. Appleton and Lange, Pp. 496.

[17]. Kim, A.H., Lee, W., Kim, M., Kim, Y. and Han, K. (2014). White blood cell differential counts in severely leukopenic samples: a comparative analysis of different solutions available in modern laboratory hematology. *Blood Res*. 49(2):120-126.

[18]. Gulack, B.C., Englum, B.R., Lo, D.D., Nussbaum, D.P., Keenan, J.E. and Scarborough, J.E. (2015). Leukopenia is associated with worse but not prohibitive outcomes following emergent abdominal surgery. J Trauma Acute Care Surg. 79 (3):437-443.

[19]. Church, J. P., Judd, J. T., Young, C. W., Kebay, J. L. and Kim. W. W. (1984). Relationships among diet constituents and specific serum clinical components of subjects eating self-selected diets. Afric. J. Clin. Nutr., 40:1338-1344.

[20]. Iheukwuemere, F., Abu, A. and Ameh, M. (2006). Effect of human menopausal gonadotropin on haematological and serum biochemical parameters of the Nigerian Indigenous chickens*. International Journal of poultry Science* 5(7):632-634.