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Effect of ethylacetate extract of *Cassytha filiformis* leaves on haematological variables in rats

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ABSTRACT

Ethylacetate extract of *Cassytha filiformis* leaves were evaluated for phytochemical components, acute and subchronic toxicity studies using standard methods. Carbohydrates, saponins, glycosides, sterols, balsams, terpenes, resins, alkaloids, and volatile oils were detected in the ethhylacetate leaf extract of *C. filiformis.* Tannins, phlobotannins, and anthraquinone were not detected in the extract. The oral LD_{50} of the extract in mice was above 5000mg/kgbw. Oral administration of the extract for 28 days to albino rats did not cause any variation in haemoglobin (Hb), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) when compared with the control group that were administered normal saline. The extract at 250mg/kgbw significantly ($P<0.05$) elevated the PCV level while 1000mg/kgbw of the extract reduced the PCV level. The WBC counts of rats were dose dependently increased. Red blood cell (RBC) counts was significantly elevated in rats. Mean cell volume (MCV) level was significantly reduced in rats fed with 1000mg/kgbw of the extract when compared with the control. Platelet counts were significantly ($P<0.05$) reduced in rats administered 500mg/kgbw and 1000mg/kgbw of the extract. Lymphocyte counts were significantly ($P<0.05$) elevated when compared with the control. Neutrophil counts were significantly reduced in all the animals exposed to different doses of the extract when compared to the control. The results of this study suggest that ethylacetate leaf extract of *C. filiformis* may contain biological active principles that have the ability to boost the immune system through increasing the population of defensive white blood cells although it possibly possesses adverse effect on platelets and neutrophil levels.

Keywords: *Cassytha filiformis*, ethylacetate leaf extract, haematology, albino rats, subchronic toxicity.

1. INTRODUCTION

Indigenous medicinal plants in Nigeria form an important component of the wealth of the country. Most of these plants have been used indiscriminately by many local population for managing various diseased states without actually knowing how relief is brought about or its safety/ toxicity risk. One of such plant is *Cassytha filiformis*. *Cassytha filiformis* (commonly called devil's gut or green dodder in

English, Rumfar Gada in Hausa, Aca-agadi in Igbo, Soko chenche in Nupe and Ominiginigini in Yoruba is a native of Florida in the United States,[1,2]. It has been widely used by many localities in Northern Nigeria in the management of diabetes, veneral discharges, haemorrhoids, cough, cancer and African trypanosomiasis [3,4]. The rationale for the use of this plant is based on long term clinical experience. Moreover, a lack of knowledge of the standardized dosage of biological substances may also be leading to toxicity [5].

Again, many people rely on herbal medicines for healthcare [6] because the other treatment options available are more expensive and are often associated with serious side effects. Therefore, information on plants' toxicity is important to the discovery of novel drugs. The present study was designed to evaluate the effects of sub chronic administration of ethylacetate extract from the leaves of *Cassytha filiformis* on albino rats by focusing on haematological indices which affect the physiological and pathophysiological status of both animals and humans [7].

Thus, the choice of haematological parameters (haemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell (neutrophils, lymphocytes, etc.) and platelets in this study.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

Fresh and healthy samples of the plant under study was obtained from Onwa Local Government, Zaria, Kaduna State, Nigeria. The plant sample was identified as *Cassytha filiformis* by two independent botanists. The plant sample was duly authenticated by an ethnobotanist of the Herbarium Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria. A voucher specimen (NIPRD/H:/6149) was deposited at the Herbarium Department, NIPRD, Idu, Abuja.

2.2. Extraction of plant materials

The leaves of *Cassytha filiformis* were used. The leaves were dried in a shade at room temperature (28±2ºC) for two weeks. Each dried sample was milled into fine powder using mortar and pestle. NIPRD protocols were employed for the extraction [8]. Fifty grams (50g) of the dried leaves was extracted with hexane (400ml) for 6 hours and then subsequently extracted with ethylacetate (400ml) for 6hours. The resulting extract was concentrated in rotavapour under reduced pressure to obtain residues (extracts). The extracts were transferred to air-tight sterile containers and stored at 4ºC. Extracts were warmed up to room temperature (28±2ºC) before use.

2.3. Phytochemical studies

Qualitative phytochemical screening was conducted to detect the presence or absence of various secondary metabolites (alkaloids, anthraquinone, balsams, carbohydrates, flavonoids, glycosides, phenols, phlobotannins, resins, saponins, steroids etc.) in ethylacetate leaf extract of *C. filiformis*. The method of Trease and Evans [9] and Medicinal Plant Research and Traditional Plant Medicine Practice, (MPR-TMP) were employed [10].

2.4. Experimental model

Healthy mice (20-30g) and albino rats (180-200g) of the same age group were used for acute and sub-chronic investigations. The animals were obtained from Animal Facility Centre, (AFC) of the Department of Pharmacology and Toxicology, NIPRD, Idu, Abuja, Nigeria. They were housed in stainless steel cages bedded with dry clean wood shavings. They were maintained at a temperature of 25±2ºC before the experiment. They were fed with standard NIPRD formulated feed and water *ad libitum*. The experimental rooms were cleaned and disinfected regularly. Soiled wood shavings were replaced often. The feed, water containers and animal cages were washed regularly. The animals were housed and cared for in accordance with good laboratory practice (GLP) regulations of WHO [11]. The principles of laboratory animal care (Natural Institute of Environmental Health and Sciences, NIEHS [12], were also followed throughout this study.

2.5. Acute toxicity studies

Effects of acute oral administration of ethylacetate leaf extract of *Cassytha filiformis* on mice was investigated by method of Aniagu et al [13]. The study was carried out in three phases. In the first phase, nine mice were randomized into 3 groups of three mice each and given 10mg/kgbw, 100mg/kgbw and 1600mg/kgbw of extract respectively. They were observed for signs of toxicity. In the second phase 1500mg/kgbw, 2000mg/kgbw and 2500mg/kgbw of the extract were administered to another fresh set of three groups of two mice each based on the result of the first phase. These mice were also observed for

signs of toxicity and mortality for the first critical 4hours and thereafter for two weeks. In the third phase, 3000mg/kgbw and 5000mg/kgbw of the extract were administered to other fresh set of two groups of two mice based on the result of the second phase. The mice were observed for signs of toxicity and mortality for the first critical 4hours and thereafter daily for two weeks.

2.6. Subchronic toxicity studies

The subchronic toxicological profiles of ethylacetate leaf extract of *Cassytha filiformis* were evaluated for 28 days in albino rats. The subchronic evaluation of ethylacetate extract was determined because the extract demonstrated marked antimicrobial activity. The methods of Aniagu et al [14] and Salawu et al [15] were employed. Twenty four rats were randomized into 4 groups of 6 rats each. The first group served as the control and received 10ml normal saline/kgbw while the rats in groups 2, 3 and 4 received 250mg/kgbw, 500mg/kgbw and 1000mg/kgbw of the extract. The rats were observed daily, before, during and after treatment for physical signs of chemical intoxication and mortality. On the 29th day of experiment, all the rats were sacrificed under dichloroethyl ether anaesthesia and blood samples were collected by cardiac puncture after opening the rats surgically. One portion was collected into k+ EDTA bottles for estimation of haematological parameters (packed cell volume (PCV), haemoglobin concentration (HB), red blood cell count (RBC), platelets, white blood cell count (WBC), and differentials (eosinophils, neutrophils, macrophages), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), using an automated haematological machine (cell – DynTM).

2.7. Statistical analysis

Data generated were expressed as mean value ± standard error of mean (SEM). Among groups, comparisons of means were performed by the analysis of variance (ANOVA) test, for statistical significance of differences at $P<0.05$. Mean values were separated by Duncan Multiple Range Test (DMRT). All data were evaluated using the statistical package SPSS version 19.0.

3. RESULTS & DISCUSSION

Carbohydrates, saponins, glycosides, sterols, basalms, terpenes, resins, alkaloids, volatile oils were detected in crude leaf extract of *Cassytha filiformis*. Tannins, phlobotannins and anthraquinone were not detected in the extract (Table 1). The LD_{50} of the extract was above 5000mg/kgbw. Ethylacetate leaf extract of *C. filiformis* did not affect the concentration of haemoglobin (HB), mean cell haemoglobin (MCH), and mean cell haemoglobin concentration (MCHC) when compared to the control (Table 2). The extract at 250mg/kgbw significantly ($p<0.05$) increased the PCV level while 1000mg/kgbw of the extract reduced it significantly ($p<0.05$). The extract dose dependently increased the WBC counts while the red blood cell (RBC) was dose independently increased. The Mean Cell Volume (MCV) count was significantly reduced in rats fed 1000mg/kgbw of the extract when compared with the control. The platelet counts was significantly ($p<0.05$) reduced in groups of rats fed 500mg/kgbw and 1000mg/kgbw of extract. The reduction was dose dependent. There was elevation of lymphocyte counts when compared with the control. The increase was not dose dependent. The neutrophil counts were reduced in extract treated groups when compared with the control.

Oral administration of ethylacetate leaf extract of *Cassytha filiformis* was accompanied by intense paw licking and sedation as well as reduced activity at all the doses of the extract tested. The animals recovered after 1-2 hours. The oral LD_{50} of the extract was estimated to be greater than 5000mg/kgbw. Ethylacetate leaf extract of *C. filiformis* is therefore regarded as relatively non-toxic acutely. This suggests that oral application of the extract may not produce severe toxic effects at doses lower than 5000mg/kgbw. Babayi et al [2] observed that the aqueous whole extract of *C. filiformis* was acutely non-toxic. This explains the safe use of the plant by the local people in traditional management of various ailments in Northern part of Nigeria.

The acute toxicity data are of limited clinical application since cumulative toxic effects do occur when consumed at low doses. Hence, sub-acute and chronic toxicity studies are almost always invaluable in evaluating the safety profile of phytomedicines. This probably explains why some authors have suggested that sub-chronic toxicity data may be needed to predict the hazard of long term, low dose exposure to a particular compound [13,14]. Thus, a 28 day oral toxicity (sub-acute) study was carried out in rats to determine the potential of ethylacetate extract of *C. filiformis* leaves to produce toxicity in man. Dose levels of 250mg/kgbw, 500mg/kgbw and 1000mg/kgbw were selected for the study.

Oral administration of ethylacetate extract of *C. filiformis* leaves for 28days was not accompanied by

death or any signs of physical toxicity in all the animals throughout the period of study. There were no changes in the nature of stool, urine and eye color of all the animals. The animals did not exhibit diarrhea, haematuria, restlessness, uncoordinated muscle movements, respiratory or cardiovascular distress during the study period.

Certain herbal preparation or conventional drugs or chemicals adversely affect various blood components. Decrease or increase in cell counts and depletion of plasma constituents or their elevation beyond reference range could equally demonstrate haematoxicity [2,15].

Haemoglobin is the iron containing oxygen transport metalloprotein in the red blood cells of all vertebrates [16]. Haemoglobin measures the total of the oxygen carrying protein in the blood which generally reflects the number of RBC in the blood. Hb and PCV are associated with total population of RBCs. Ethylacetate leaf extract of *C. filiformis* did not affect the Hb concentration of rats throughout the experimental period. This is in agreement with Ajibade et al[17] who observed the non-significant change in Hb concentration in rats fed with methanolic extract of *Moringa oleifera* seed (160mg/kgbw, 400mg/kgbw and 800mg/kgbw) and concluded that the extract may not contain toxic substances that can cause anemic condition in rats. This observation is also in agreement with the report of John [18] who also did not observe any toxic effect in Hb level in wistar rats fed with *M.oleifera.* Therefore, the ethylacetate extract of *C.filiformis* is not likely to adversely affect the oxygen carrying capacity of the blood of the animals.

PCV is the percentage of red blood cells in the blood circulated around the body. It is a point of reference of the capability of RBCs to deliver oxygen to tissues. Reference range for PCV is 34-57% [19]. An increased PCV value indicates abnormal increase in RBC production or dehydration while a low PCV depicts loss of RBC as a result of blood loss, failure of bone marrow production and cell destruction. Since PCV levels reflect the efficiency and extent of oxygen uptake and transfer to tissues, the observed reduction in PCV values in rats treated with 1000mg/kgbw of ethylacetate leaf extract of *C. filiformis* in the present study suggest different levels of disturbance in osmoregulatory system of the blood cells or an oxidative injury to the cell membrane. This result is consistent with the findings of Ladokun et al [20] who observed reduction in PCV and RBC in osmoregulatory system of blood cells of albino rats fed with aqueous extracts of *Viscum album.* Mbajiorgu et al [21] observed that changes in PCV levels affect the extent and efficiency of oxygen uptake and transfer to tissues and significantly a reduction in the body metabolic activity. Furthermore, 250mg/kgbw of extract increased the PCV level of the rats. This may be due to an abnormal increase in RBC production. The ethylacetate extract of *C. filiformis* may contain biological principles which are capable of reducing or increasing PCV levels of rats.

Ethylacetate extract of *C. filiformis* leaves dose dependently elevated WBC counts. This could be due to the fact that the extract contained biological active principles (quinones, terpenoids, phenols etc.) that have the ability to boost the immune system through increasing the population of defensive white blood cells. Such effects may also be due to increase in vascular permeability [22]. Again, this could be an advantage to diabetics who are more prone to infections. Rajagopal et al [23] observed that various plant extracts have immunostimulatory activity as evidenced by increased proliferation of lymphocytes and production of interleukin-2. Wagner et al [24] reported that various plants derived metabolites (alkaloids, quinones, terpenoids, phenol, carboxylic acids, polysaccharides, and glycoproteins) possess immune-stimulatory activity.

Adedapo et al [25] and Mohajeri et al [26] reported that increased WBC count is helpful in boosting the immune system. However, the findings of the present study is not in agreement with Adebayo et al[27] who reported that ethanolic extract of *Bougainvillea spectabilis* decreased the count of WBC.

MCV, MCH and red blood cell indices provide information on the physical characteristics of the red blood cells. MCV is a measurement of the average size of a single red blood cell while MCH measures the average weight of hemoglobin inside a single red blood cell [28]. MCV is useful in the differential diagnosis of anaemia.

Ethylacetate leaf extract of *C. filiform* did not affect the MCH, MCHC of rats. Yakubu et al [24] made a similar observation to the findings of the present study when rats were fed with aqueous extract of *Fadogia agrestis* stem (18mg/kgbw, 50mg/kgbw and 100mg/kgbw). He observed that *F. agrestis* did not produce any significant change ($p>0.05$) on RBC and factors relating to it (Hb, PCV, MCV, MCH and MCHC) throughout their experimental period (21 days).

The results of the present study is also in agreement with the observations made by Adebayo et al[27] on administration of ethanolic extract of *B. spectabilis* leaves showing no significant effect on MCH and MCHC when compared with the control. Bunchareon et al[29] observation on non-significant changes in MCH and MCHC levels in rats fed with ethanolic extract of *Stemona aphylla* (300mg/kgbw and 500mg/kgbw) for 45 days is in agreement with the findings of the present study.

This is an indication that there was no destruction of matured RBCs (erythropoiesis). It further showed that the extract does not have potential to stimulate erythropoietin release in the kidney which is the humoral regulator of RBC. The non-significant effects of ethylacetate extract of *C. filiformis* leaves on RBC indices suggest that there was no effect on the average size of RBC (microcyctes) and also in the haemoglobin weight per RBC. This means that ethylacetate extract of *C. filiformis* does not possess any potential of inducing anemia throughout the 28 days of administration.

Platelets are determinants of blood viscosity which correlates positively to blood pressure. Platelets (thrombocytes) are the smallest formed elements of the blood. They are vital to coagulation of the blood to prevent excessive bleeding. Platelets increase may be due to stimulatory effect on thrombopoietin [30]. A decreased number of platelets (thrombocytopenia) may indicate an immune system failure, drug reactions, B12, folic acid deficiency or bleeding [31]. The significant increase in platelet counts at low dose (250mg/kgbw) and decrease in platelet counts at high dose (1000mg/kgbw) of ethylacetate leaf extract of *C. filiformis* agrees with the observation of Yakubu et al [24] who studied the effect of aqueous extract of *Fadigia agretis* stem in rats. The result of the present study indicate that the medicinal plant extract may contain bioactive principles that are capable of increasing and reducing platelets count of the animals [16].

Elevation of lymphocytes reflects possible leukopoietic and immunomodulatory effects of ethylacetate extract of *C. filiformis* leaves in rats. Yakubu et al [24] reported that aqueous extract of *Fadigia agretis* increased the percentage lymphocytes significantly throughout their experimental period at all the doses (18mg/kgbw, 50mg/kgbw and 100mg/kgbw). Bunchareon et al [29] also observed elevation of lymphocytes following oral administration of *Stemona aphylla* root extract in rats for 45 consecutive days. According to Adeneye [32] and Palani et al [33], plant extracts that produce elevation in lymphocytes counts may contain bioactive ingredients with haematopoietins synthesis or release from haematopoietic organs such as kidney and liver. Bunchareon et al [29] also adduced elevated lymphocytes count in treated groups to chronic inflammation of liver and kidney of rats after administration of *S. aphylla* extract. Therefore, ethylacetate extract have immunostimulatory properties influencing cell mediated immune system.

The significant reduction in percentage of neutrophils observed in the present study may be due to the fact that the extract may possess some anti-neutrophilic activity [24]. High level of neutrophil indicate active infection while low count may indicate impaired immune system or suppression in bone marrow. The reference range for neutrophils is 56 [34]. According to Dacie and Lewis [35] and Yakubu et al [24], the reduction in neutrophils may be adduced to impairment in the ability to phagocytose (cellular ingestion of offending agents). Ajibade et al [17] attributed significant decrease in neutrophil counts to suppression of leucopoiesis in the bone marrow. According to Afolayan and Yakubu [36], this result may have consequential effect on the immune system and phagocytic activity of the blood cells of the animals. The results of the present study on reduced neutrophil count contradicts the report of Swenson and Reece [37] who reported that toxic plants do not produce a direct effect on white blood cell and its functional indices.

Reduction in neutrophils counts in the present investigation was however compensated by an increase in lymphocyte counts. Lymphocytes and neutrophils are main defender of the body against infection and antigens [34,38,39]. Oral administration of ethylacetate leaf extract appeared to exhibit stimulatory effects on cells of the immune system.

Table 1. Phytochemical components of the ethyacetate crude extract of *C. filiformis*

Phytochemical components)	Extract
Alkaloids	-
Anthraquinone	+
Balsams	+
Carbohydrates	-
Flavonoids	+
Glycosides	+
Phenols	+
Phlobotannins	-
Resins	+
Saponins	+
Steroids	+
Tannin	-
Terpenes	+
Volatile oil	+

+: present, -: absent

Table 2. Effect of ethylacetate leaf extract of *C. filiformis* on haematological parameters of rats

	Treatment(mg/kgbw)			
	Control	**250**	**500**	**1000**
HB(g/dl)	10.78±0.55	11.71±0.99	11.32±0.51	10.80±60.00
PCV (%)	38.21±1.34	43.17±1.59*	37.00±2.11	35.00±1.00*
WBC(×10^9/l)	11.42±0.68	16.97±1.22*	21.15±3.18*	22.58±1.52*
RBC(×10^9/l)	6.10±0.55	11.71±0.99*	11.32±0.51*	10.80±0.60*
MCH(Pc)	17.60±0.10	17.47±0.96	17.90±0.46	18.13±0.41
MCH(g/dl)	29.08±0.32	30.73±1.08	30.72±0.46	31.75±0.28
MCV(fl)	60.23±0.79	59.35±0.78	59.47±1.60	56.83±1.62*
Platelets(×10)	773.33±23.17	734.33±65.02	507.5±48.18*	483.33±49.90*
LYM (%)	72.00±4.51	77.00±2.41*	75.0±1.57*	80.83±1.40*
NEU (%)	30.00±2.15	22.50±1.31*	22.50±1.71*	14.50±1.89

*:Significantly different from the control at $P<0.05$, n=6

Hb: Hemoglobin concentration, MCV: Mean Cell Volume, WBC: White Blood Cell, LYM: Lymphocyte count, NEU: Neutrophil count, g/dl: gram per deciliter, MCH: Mean Cell Hemoglobin, PCV: Packed Cell Volume, fl: femto litre, MCHC: Mean Cell Hemoglobin Concentration, RBC: Red Blood Cell, Mg/Kg.bw: Milligram per kilogram body weight of animals

Conflicts of Interest

There are no conflicts of interest.

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References

1. Abdullahi, M., Mohammed, G. and Abdulkadir, N.U. (2003). *Medicinal and Economic Plants of Nupeland*. Jubes-Evans Publications, Bida, Nigeria, pp.140.
2. Babayi, H.M, Ijah, U.J.J., Abalaka, J.A., Okogun, J.I., Salawu, O.A., Akumka, D.D., Adamu, A., Zarma, S., Adzu, B.A., Abdulmumuni, S., Kolo, I., Elisha, B., Zakariya, S. and Inyang, U.S. (2007). Effects of oral administration of aqueous whole extract of *Cassytha filiformis* on hematograms and plasma biochemical parameters in rats. *Journal of Medical Toxicology,* 3(4) 146-156.
3. Neuwinger, H.D. (2002). African traditional medicine. A dictionary of plants'use and applications. *Medicinal Pharmacology* 99: 1-12.
4. Lerclercq, J.Q., Hoet, S., Block, S., Wautier, M.C. and Stevingny, C. (2004). Studies on *Cassytha filiformis* from Benin: Isolation, biological activites and quantification of aporphines. *Proceedings of Bioresources towards Drug Discovery and Development*, pp.81-106.
5. Zhu, Y.Z., Huang, S.H., Tan, B.K.H, Sun, J. Whiteman, M., Zhu Y.C. (2004). Antioxidants in Chinese herbal medicines: a biochemical perspective. *Natural Products Responses.* 21:478-489.
6. Martins, M.R., Arantes, S., and Candeias, F., Tinico, M.T. and Cons, J. (2014). Antioxidant and toxicological properties of *Schinus molle L.* essential oils. *Journal of Ethnopharmacology* 151:1
7. Adeneye AA (2008). Haematopoetic effect of methanol seed extract of Citrus paradise Macfad (grape fruit) in Wistar rats. *Biomedical Resources.* 19(1):23-26.
8. National Institute for Pharmaceutical Research and Development, NIPRD (2004). Extraction of Medicinal plants. Idu, Abuja, Nigeria.
9. Trease, G.E., and Evans, W.C. (1989). *Pharmacology* (13th ed). Bailliere Tindall, Britain: English Language Book Society. Pp. 378-480.
10. Medicinal Plant Research and Traditional Medicinal Practice, MPR-TMP (2006). Phytochemical Screening and Isolation of Active Components of Medicinal Plants Extracts. NIPRD, Abuja, Nigeria.
11. WHO (1998). Basic principles of good laboratory practice. http://www.who.int /tdr/publications (accessed May, 2017).
12. Natural Institute of Enviromental and Health Science, NIEHS. (1985). Respect for Life. http://www.nichsnih.gov/oc/factsheets/ wrl.studybgn. (Accessed May, 2017).
13. Aniagu, S.O., Nwinyl, F.C., Akumka, D.D., Ajoku, G.A., Dzarma, S., Izebe, K.S., Ditse, M., Nwaneri, P.E.C., Wambebe, C. and Gamaniel, K. (2005). Toxicity studies in rats fed nature cure bitters. *African Journal of Biotechnology*, 4(1):72-78.
14. Salawu, O.A., Tijani, A.Y., Obidike, I.C., Tags, S.Z., Dzarma, S., Osunkwo, U.A., Nelson, O.O., Okogun, J.I. and Inyank, U.S. (2009a). Safety evaluation of NIPRD AD-1 in Rats: An antidiabetic phytomedicine. *Journal of Phytomedicine and Therapeutics* 13:1-10.
15. Tan, V.P., Maurice, B., George, E., Enow, O., Francois-Xavier, E. and Bitolong, P. (2007). Acute and subacute toxicity profile of the aqueous stembark extract of *Enantia chlorantha* (annonaeceae) in laboratory animals. *Pharmacology Online*, 1:304.
16. Salawu, O.A., Tijani, A.Y., Obidike, I.C., Tags, S.Z., Dzarma, S., Osunkwo, U.A., Nelson, O.O., Okogun, J.I. and Inyank, U.S. (2009b). Safety evaluation of NIPRD AD-1 in Rats: An antidiabetic phytomedicine. *Journal of Phytomedicine and Therapeutics* 13:1-10.
17. Dioka, C., Orisakwe, O.E., Afonne, O.J., Agbasi, P.U., Akumka, D.D. and Okonkwo, C.J. (2002). Investigation into the haematologic and hepatoxic

effects of rinba-cin in rats. *Journal of Health Science.* 48(5):393–398.
18. Wolfsthal, S.D. (2012). *NMS medicine*. Philadelphia: Wolters Kulwer Health/Lippincott Willams and Wilkins. Pp. 115.
19. Ajibade, T.O., Olayemi, F. and Arowolo, R.O.A. (2012).The haematological and biochemical effects of methanol extract of the seeds of *Moringa oleifera* in rats. *Journal of Medical Plant Resources* 6(4): 615-621.
20. John, S.A.A. (1988). Using Moringa oleifera seeds as coagulant in developing countries. *Journal of American. Water Works Associations* 6: 43-50.
21. Probst, R.J., Lim, J.M., Bird, D.N., Pole, G.L., Sato, A.K. and Claybaugh, J.R. (2006). Gender differences in the blood volume of conscious Sprague-Dawley rats. *Journal of the American Association for Laboratory Animal Science,* 45(2), 49-52.
22. Ladokun, O., Ojezele, M., and Arojojoye, O. (2015). Comparative study on the effects of aqueous extracts of *Viscum album* (mistletoe) from three host plants on hematological parameters in albino rats. *African Health Sciences,* 15(2), 606-612.
23. Mbajirogu, E., Air, T., Volk, W., Alberet, M. and Debusho, L. (2007). Haematological profile of male rats treated with ethanol and/or chloroquine and fed normal or low protein diet. *The Internet Journal of Haematology,* 3(1):1-11.
24. Yakubu, M.T., Akanji, M.A. and Oladiji, T.A. (2005). Haematological evaluation in male albino rats following chronic administration of aqueous extract of *Fadogia agretis* stem. *Pharmacognosy Magazine*, 973:34-38.
25. Adedapo AA, Abatan MO, Olorunsogo OO (2007). Effects of some plants of the spurge family on the haematological and biochemical parameters of rats. *Veterinary Archives.* 77: 29-38.
26. Mohajeri, D., Mousavi, G, and Mesgari, M. (2007). Subacute toxicity of *Crocus sativus* L. (saffon) stigma ethanolic extracts in rats. *American Journal of Pharmacology and Toxicology,* 2:189-193.
27. Adebayo, J.O., Adesokan, A.A., Olatunji, L.A., Buoro, D.O. and Soladoye A.O. (2005). Effect of ethanolic extract of *Bougainvillea spectabilis* leaves on haematological and serum lipid variables in rats. *Bioline Biokemistri.* 17:45-50.
28. Andrews, R. (2010). Blood tests & lab analysis: How it works and what you need to know. Retrieved from Precision Nutrition website; http://www.precisionnutrition.com/all-about-blood-work on May, 2017.
29. Buncharoen, W., Saenphet, S., homdej, S. and Saenphet, K. (2012). Evaluation of biochemical, hematological and histopathological parameters of albino rats treated with *Stemona aphylla* Craib. Extract. *Journal of Medicinal Plant Research* 6 (27) 4429-4435.
30. Ezumi, Y., Takayama, H., and Okuma, M. Thrombopoietin, c-Mpl ligand, induces tyrosine phosphorylation of Tyk2, JAK2, and STAT3, and enhances agonists-induced aggregation in platelets in vitro. *FEBS Letters*. 1995; 374(1):48–52.
31. Welch, K.M.A., Reis, D.J., Caplan, R.J., & Siesjo, B.K. (1997). *Primer on cerebrovascular diseases.* San Diego, CA: *Academic Press.* pp.67.
32. Adeneye, A.A (2008). Haematopoetic effect of methanol seed extract of *Citrus paradise Macfad* (grape fruit) in Wistar rats. *Biomedical Resources* 19(1):23-26.
33. Palani S, Senthilkumar B, Praveen R, Kumar P, Devi K, Venkatesan D, Sathendra ER (2009). Effect of the ethanolic extract of Indigofera barberi (L) in acute acetaminophen -induced nephrotoxic rats. *Advanced Biotechnology* 25:28-31.
34. McPherson, R., and Pincus, M. (2011). *Henry's Clinical Diagnosis and Management by Laboratory Methods* (22nd Ed.) Philadelphia, PA: Elsevier Sanders. Pp.133-137.
35. Dacie, J. & Lewis, S. (1991). *Practical Hematology,* (7th Ed.). Churchill Livingston. Edingburgh.
36. Afolayan AJ, Yakubu MT (2009). Effect of Bulbine natalensis Baker stem extract on the functional indices and histology of the liver and kidney of male Wistar rats. *Journal of Medical Food,* 12: 814-820.

37. Swenson, M.J., Reece, O.W. (1993). Duke′s Physiology of Domestic Animals. Ithaca: Comstock Publishing Associates, pp. 312-315.
38. Campbell TW (1996). Clinical pathology. In: Mader DR (ed) Reptile Medicine and Surgery. WB Saunders Company, Philadelphia, PA, U.S.A. pp.248-257.
39. McKnight, D.C., Mills, R.G., Bray, J.J. and Crag P.A (1999). Human Physiology. 4th edition. Churchill Livingstone. pp, 290-294.