

# Determination of Hematological Effects of Methanolic Leaf Extract of *Vernonia lasiopus* in Normal Mice

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#### Abstract

Hematological disorders have attained epidemic proportions worldwide today. As a result, many people turn to medicinal plants for treatment thereby boosting and enhancing health because professional care is not immediately available, is too inconvenient, costly and time consuming. Certain medicinal plants are believed to promote positive health and maintain organic resistance against infection. The use of medicinal plants which are readily available and arguably efficacious would therefore over a better and affordable alternative for boosting and enhancing health. Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal. Various chemical constituents of Vernonia lasiopus are believed to possess therapeutic effects on hematological parameters. However, these effects have not been subjected to systematic studies to substantiate the therapeutic claims made regarding their clinical utility. This study was designed to investigate the hematological effects of V. lasiopus (O. Hoffman) in normal mice. The experimental groups were treated with leaf extracts at concentration of 50 mg/kg and 100 mg/kg orally once per two days for a period of fourteen days. Hematological parameters and indices were determined from unclotted blood samples using standard protocols. Presence of various types of phytochemicals was assessed using standard procedures. The leaf extract of V. lasiopus (O. Hoffman) induced changes in erythrocytes and related parameter profiles, total and differential WBC counts, platelets and their related parameters in normal mice at the two tested dose levels of 50 mg/kgbw and 100 mg/kgbw. Further, the phytochemical screening results showed that the leaf extract of V. lasiopus (O. Hoffman) have phytochemicals associated with erythropoietin promoting activity, immunostimulatory activities and thrombopoietin stimulation.

**Keywords:** Hematological disorders; *Vernonia lasiopus*; Leaf extract; Erythropoietin promoting activity; Immunostimulatory activities; Thrombopoietin stimulation

#### Introduction

Blood is a specialized body fluid that delivers necessary substances to the cells such as nutrients and oxygen and transports waste products away from the cells [1]. It accounts for 7% of human body weight with an average density of approximately 1060 kg/m<sup>3</sup> and is composed of plasma and several kinds of cells which include erythrocytes, leucocytes and thrombocytes [2].

Erythrocyte have three main functions; to distribute oxygen to the periphery from the lungs through the pulmonary capillaries, remove carbon dioxide from the tissues back to the lungs through the systemic capillaries, and ensure that the acidic and basic values of the body are normal [3].

Leucocytes (white blood cells) are the main cells of the immune system that provide either innate or specific adaptive immunity. They are of five different kinds; neutrophils, monocytes, lymphocytes, eosinophils and basophils. Leucocytes are further classified into lymphocytes, granulocytes, monocytes, and natural killer cells [4].

Platelets are involved in many pathophysiological processes including hemostasis and thrombosis, clot retraction, vessel constriction and repair, inflammation including promotion of atherosclerosis, host defense and even tumor growth/ metastasis [5].

Assessment of hematological parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts on the blood constituents of an animal [6].

According to WHO criteria, anemia in adults is defined as hemoglobin level of 13 g/dl or lower for men and 12 g/dl or lower for

women [7]. Treatment is usually aimed at correcting the underlying abnormality.

Acquired anemia associated with hematopoietic abnormalities or inherited anemia may require blood transfusions when symptoms arise due to decreased oxygen delivery to the tissues [8].

Treatment of anemia with Erythropoiesis-Stimulating Agents (ESA) effectively corrects the condition in patients with chronic kidney disease. Administration of erythropoietin to improve hemoglobin is recommended in solid organ recipients patients [9].

Stopping the administration of drugs such as methyldopa, some anti-biotics and hydrochlorothiazide that cause drug related anemia corrects the condition [10].

In cases of aplastic anemia, use of Anti-Thymocyte Globulin (ATG), or allogenic bone marrow transplantation and hemopoietic growth factors such as Granulocyte Colony Stimulating Factor (G-CSF) is advised [11].

Neutropenia occurs in cases of reduced production of white blood cells or increased utilization and destruction, or both. In situations

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where the etiological factor can be identified and rectified like the case of drugs, folic acid deficiency, treatment should be instituted without delay [12].

Neutropenia resulting from chemotherapy or radiation may, in specific instances, can be treated with cytokines such as granulocyte colony—stimulating factor or granulocyte-macrophage colony—stimulating factor [13].

Severe congenital neutropenia whereby the patient fails is to respond to granulocyte colony-stimulating factor treatment, hematopoietic stem cell transplantation can successfully be used in treatment [14].

Thrombocytopenia means a reduction in the platelet count below the normal lower limit, which is usually defined as  $150 \times 10^{9}$ /L [15]. This can be caused by reduction in platelet production, reduction in platelet survival, and dilution of platelet numbers resulting from transfusion of platelet-poor blood [16].

Platelet function disorders may be inherited including disorders of platelet adhesion, idiopathic alpha, disorders of platelet granules [17] or acquired platelet function disorders including medication and chemicals, chronic kidney disease and myeloproliferative disorders [18].

In case of severe thrombocytopenia or evidence of hemorrhage, a patient should receive immediate attention by undertaking platelet transfusion [19].

Immune thrombocytopenia initial management is undertaken with corticosteroids. Short "pulses" of dexamethasone have been found to be very effective [20] while emergent plasma exchange is the cornerstone of TTP treatment [21].

Most conventional ways of managing anemia, neutropenia and thrombocytopenia may be costly, have undesired side effects, painful to the patients or are not easily accessible.

All these limitations involved in the use of conventional methods to manage hematological disorders require the need to look for alternative safer and effective remedies devoid of the above shortcomings to contain and manage these hematological disorders.

It is acknowledged world-wide that traditional medicine can be explored and exploited to be used along-side synthetic pharmaceutical products for enhanced health management [22]. Certain medicinal plants are believed to promote positive health and maintain organic resistance against infection by re-establishing body equilibrium and conditioning the body tissues [23].

*V. lasiopus* (O. Hoffman) pounded leaves and paste has been traditionally used to treat worms in infected wounds [24], hepatitis and kids constipation [25,26]. Stem decoction has been used in treatment of malaria, worms and non-bacterial infections [27], epilepsy, indigestion and during childbirth [28].

Therefore, this study was aimed at evaluating the hematological effects of methanolic extracts of *Vernonia lasiopus* (O. Hoffman) on normal mice as a preliminary step towards development of a more efficacious plant-derived agent to manage hematological disorders and boost health.

#### **Materials and Methods**

#### Collection and preparation of sample materials

Fresh leaves of V. lasiopus were collected from Siakago division,

Mbeere North sub-county, Embu County, Kenya. Fresh leaves were identified with the help of local herbalists. The information gathered included vernacular names, plant parts used and the ailment treated. The samples were properly sorted out and transported in polythene bags to Kenyatta University, Biochemistry and Biotechnology laboratories for drying and crushing. The identity of each of the plants was authenticated by a taxonomist in the Department of Plant and Microbial Sciences, Kenyatta University's Herbarium for future reference. The fresh leaves of *V. lasiopus* (O. Hoffman) were cleaned with tap water and dried under shade then pulverized to powder using an electrical blender.

#### Extraction

500 mg of the leaf powder was weighed and soaked in 1 L of methanol in a conical flask. The flask containing the leaves was shaken, corked and left to stand for 48 hrs at room temperature. The mixture was then filtered using Whatman No.1 filter paper and the filtrate Rota-evaporated to dryness at 65°C to recover the extract. The concentrate was put in an air tight container and stored at 4°C before use in bioassay studies.

#### Experimental animals and design

Three to four week old healthy male Swiss albino mice weighing an average of 20 g were used in this study. They were bred in the animal house of the Department of Biochemistry and Biotechnology, Kenyatta University. The mice were housed in polypropylene cages, maintained under standard laboratory conditions of 12 hour light and dark sequence, at ambient temperature of  $25 \pm 2^{\circ}$ C and 35-60% humidity. The animals were fed with standard mice pellets obtained from Unga Feeds Limited, Kenya, and water *ad libitum*. Ethical guidelines and procedures for handling experimental animals were followed.

The animal were randomly divided into 2 groups (Group A and B) of 5 mice each. Animals in Group A and B were orally administered with 50 mg/kgbw and 100 mg/kgbw of methanolic extract respectively at intervals of two days for 14 days. The extract was administered using intragastric gavage technique. Blood from both groups of mice was taken before the commencement of the first oral administration, then repeated on the seventh day and at the end of the experimental period. During this period, mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behavior and/or mortality.

#### Preparation of extracts doses for administration

The dose level of 50 mg/kgbw was prepared by dissolving 0.042 g of the extract in 4.2 ml of 30% dimethylsulfoxide and topping up to 10ml with distilled water, while the dose level of 100 mg/kgbw was prepared by dissolving 0.084 g in 4.2 ml of 30% dimethylsulfoxide and topping up to 10 ml with distilled water.

#### **Collection of blood samples**

Blood samples were collected at the start of the experiment, then on the 7<sup>th</sup> day and finally on the 14<sup>th</sup> day from the tails of mice for the determination of hematological parameters. The tails were first sterilized by swabbing with 70% ethanol and then the tip of the tails snipped with sterile scissors. Bleeding was enhanced by gently milking the tail from the body towards the tip. Blood of approximately 0.2 ml was drawn into bottles containing anticoagulant (EDTA) shaken and taken for hematological parameter assessment. On the 14<sup>th</sup> day the animals were euthanized by use of chloroform. Citation: Muriithi NJ, Maina GS, Maina MB, Kiambi MJ, Kelvin JK, et al. (2015) Determination of Hematological Effects of Methanolic Leaf Extract of Vernonia lasiopus in Normal Mice. J Blood Lymph 5: 139. doi:10.4172/2165-7831.1000139

#### Determination of hematological parameters

Hematological parameters and indices were determined from unclotted blood samples using standard protocols [29]. Erythrocytes, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width and platelets, plateletcrit, mean platelet volume and platelet distribution width, were determined using the Coulter Counter System (Beckman Coulter\*, ThermoFisher, UK). Air-dried thin blood films stained with Giemsa stain were examined microscopically using magnification x200 and x400 for differential white blood cell counts.

#### Qualitative phytochemical screening

The crude extracts obtained were subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described by [30,31]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested included alkaloids, flavonoids, phenolics, saponins, terpenoids, cardiac glycosides, steroids and tannins.

#### Data management and analysis

Experimental data on different hematological parameters was obtained from all the animals on the 1<sup>st</sup> day and compared with the 7<sup>th</sup> and 14<sup>th</sup> day for the two dose levels. It was recorded and tabulated on a broad sheet using Ms Excel program. The results were expressed as mean  $\pm$  Standard Error of Mean (SEM) for analysis. Statistical significance of difference among the two groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's tests to separate the means and obtain the specific significant differences among the different groups. The values of P  $\leq$  0.01 were considered to be significant. Analysis of the data was done using Minitab statistical software.

#### Results

## Effects of methanolic leaf extract of *V. Lasiopus* (O. Hoffman) on erythrocytic parameter profiles in normal mice.

Methanolic leaf extract of *V. lasiopus* (O. Hoffman) induced changes in erythrocytes and related parameter profiles in normal mice (Table 1). Seven days after administration of the extracts at the dose levels of 50 mg/kgbw and 100 mg/kgbw, there was significant increase in erythrocytes and hematocrit counts (p<0.01; Table 1). The two dose levels had no significant effects on Hb, MCV, MCH, MCHC and RDW profiles in normal mice (P>0.01; Table 1).

### Effects of methanolic leaf extract of *V. lasiopus* (O. Hoffman) in total WBC and differential WBC counts in normal mice

Methanolic leaf extract of *V. lasiopus* (O. Hoffman) induced changes in total differential WBC counts in normal mice (Table 2). The dose level of 50 mg/kgbw did not have any significant effect on total WBC, monocytes and eosinophils counts (p>0.01; Table 2), but it caused a significant increase in neutrophils, lymphocytes and basophils counts (p<0.01; Table 2). The dose level of 100mg/kgbw caused a significant increase in total WBC, neutrophils, and basophils counts (p<0.01; Table 2) but had no significant effect on monocytes and eosinophils counts (p<0.01; Table 2).

## Effects of methanolic leaf extracts of *V. lasiopus* (O. Hoffman) in platelets and their related parameter profiles in normal mice

The Methanolic leave extract of *V. lasiopus* (O. Hoffman) also induced changes in platelets and their related parameter profiles in normal mice (Table 3). The dose level of 50 mg/kgbw caused a significant increase in platelets and MPV levels after seven days of administration (p<0.01; Table 3) but did not have a significant effect in plateletcrit and PDW (p>0.01; Table 3). The dose level of 100mg/kgbw caused a significant increase in platelet and plateletcrit after fourteen

Parameters	50 mg/kgbw			100 mg/kgbw		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Erythrocytes	3.40 ± 0.08	5.57 ± 0.10	5.83 ± 0.09	3.84 ± 0.15	5.30 ± 0.08	5.72 ± 0.13
Hb	6.74 ± 0.44	7.91 ± 0.6	8.12 ± 0.90	6.11 ± 0.12	7.21 ± 0.25	8.65 ± 0.5
Hematocrit	16.74 ± 0.86	26.05 ± 1.18	30.27 ± 0.43	15.75 ± 0.69	23.81 ± 1.5	26.44 ± 0.86
MCV	51.82 ± 1.63	49.28 ± 1.46	54.77 ± 0.54	53.20 ± 0.00	51.82 ± 3.23	55.43 ± 2.61
МСН	13.96 ± 0.47	14.82 ± 0.40	15.08 ± 0.4	13.76 ± 0.50	14.62 ± 0.34	15.13 ± 0.23
МСНС	27.08 ± 1.26	30.10 ± 0.51	28.85 ± 0.59	26.86 ± 86	28.50 ± 1.19	28.18 ± 0.98
RDW	15.64 ± 0.56	14.92 ± 0.39	16.76 ± 0.47	16.22 ± 0.65	15.54 ± 0.65	15.54 ± 0.65

All values are expressed as mean ± SEM for five animals per group. Values for each parameter are compared among day 0, 7 and 14 for each dose by ANOVA and Tukey's post hoc test.

Table 1: Effects of Methanolic leaf extract of V. lasiopus (O. Hoffman) on Erythrocytes and related parameter profiles in normal mice.

Parameters	50 mg/kgbw			100 mg/kgbw		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
WBC	5.22 ± 0.15	6.72 ± 0.56	7.20 ± 0.37	5.56 ± 0.24	7.48 ± 0.12	7.92 ± 0.12
Neutrophils	0.78 ± 0.04	1.34 ± 0.06	1.92 ± 0.06	0.76 ± 0.16	1.42 ± 0.11	1.86 ± 0.11
Lymphocytes	3.22 ± 0.09	3.86 ± 0.10	4.16 ± 0.10	4.04 ± 0.09	3.98 ± 0.04	4.12 ± 0.06
Monocytes	0.00 ± 0.0	0.00 ± 0.00	0.06 ± 0.0	0.00 ± 0.00	010 ± 0.08	0.24 ± 0.09
Eosinophils	0.022 ± 0.05	0.02 ± 0.02	0.40 ± 0.13	0.12 ± 0.06	0.01 ± 0.03	0.22 ± 0.07
Basophils	0.14 ± 0.05	0.70 ± 0.13	1.14 ± 0.02	0.20 ± 0.03	0.60 ± 0.11	1.12 ± 0.08

All values are expressed as mean ± SEM for five animals per group. Values for each parameter are compared among day 0, 7 and 14 for each dose by ANOVA and Tukey's post hoc test.

Table 2: Effects of Methanolic leaf extract of V. lasiopus (O. Hoffman) on White blood cells and their differentials parameter profiles in normal mice.

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Parameters	50 mg/kgbw			100 mg/kgbw		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Platelets	206.60 ± 4.25	368.40 ± 7.81	423.80 ± 19.4	203.6 ± 13.9	257.80 ± 9.98	382.00 ± 4.83
Plateletcrit	0.04 ± 0.00	0.08 ± 0.01	0.31 ± 0.20	0.04 ± 0.0	0.06 ± 0.01	0.14 ± 0.01
MPV	1.72 ± 0.12	2.06 ± 0.14	2.64 ± 0.12	1.80 ± 0.05	2.64 ± 0.13	3.36 ± 0.15
PDW	14.86 ± 0.37	14.68 ± 0.18	15.18 ± 0.40	15.08 ± 0.39	14.60 ± 0.56	15.75 ± 0.35

All values are expressed as mean ± SEM for five animals per group. Values for each parameter are compared among day 0, 7 and 14 for each dose by ANOVA and Tukey's post hoc test.

Table 3: Effects of Methanolic leaf extract of V. lasiopus (O. Hoffman) on platelets and their related parameter profiles in normal mice.

days of oral administration of the extract (p<0.01; Table 3). It also caused a significant increase in MPV after seven and fourteen days of administration (p<0.01; Table 3) but did not have any significant effect on PDW (p>0.01; Table 3).

#### Phytochemical screening

Qualitative phytochemical screening of the Methanolic leaf extracts of *V. lasiopus* (O. Hoffman) indicated presence of alkaloids, flavonoids, saponins, phenolics and tannins (Table 4).

#### Discussion

Blood parameters are key factors in diagnosing the actual physiological status of organisms. An organism must keep its blood composition and constituent relatively constant under natural conditions to function properly [32]. The present study showed that methanolic leaf extracts of *V. lasiopus* (O. Hoffman) demonstrated varying degrees of hematological changes at the dose levels of 50 mg/ kgbw and 100 mg/kgbw.

The significant increase in erythrocytes and hematocrit counts after oral administration of methanolic extracts of *V. lasiopus* (O. Hoffman) suggests that the extracts may contain compounds and phytochemicals that stimulate the formation or secretion of erythropoietin in the stem cells of normal mice. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells [33]. Erythropoietin affects the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since red blood cells and hemoglobin are very important in transferring respiratory gases [34,35].

It may also suggest that the extracts can cause polychethermia. Previous studies have indicated that an increase in the count of erythrocytes and PCV is suggestive of polycythermia and positive erythropoiesis [36-39]. Moreover, studies by [40] suggested that the leaves of *Peristrophe bicalculata* (Retz) are capable of increasing erythrocyte counts in experimental animals. They confirmed the use of *Peristrophe bicalculata* (Retz) leaves in restoring lost blood during

Phytochemicals	V. lasiopus (O. Hoffman)		
Alkaloids	+		
Flavonoids	+		
Steroids	-		
Saponins	+		
Cardiac glycosides	-		
Phenolics	+		
Terpenoids	-		
Tannins	+		

Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign.

 Table 4: Qualitative phytochemical screening of Methanolic leaf extract of V. lasiopus.

excessive bleeding [41], who worked on *Baphia nitida* (Lodd) also reported similar results. Therefore, the methanolic extracts of *V. lasiopus* (O. Hoffman) and *S. incanum* (Linn) can be used to restore lost blood during excessive bleeding.

As [42] observed, the mechanism leading to the increase in erythrocyte count is probably mediated by the anti-oxidant property of the extracts. The presence of antioxidant phytochemicals like flavonoids and tannins in the methanolic extracts of *V. lasiopus* (O. Hoffman) may be responsible for the haemopoietic stimulating effects. This is in line with previous research that showed that prophylactic and therapeutic oral administration of anti-oxidant supplements in plant extracts significantly increased cells of hemopoietic origin in animals exposed to potentially lethal dose of radiation [42]. Flavonoids, tannins and terpenes have been found to protect erythrocytes from oxidative damage [43]. Further [44,45] reported that flavonoids have various benefits for human health due to its anti-oxidant and free-radical scavenging activities as well as anti-inflammatory, antiviral, and anticancer properties.

Since MCHC, MCH and MCV profiles relate to individual red blood cells while hemoglobin and hematocrit profiles relate to the total population of red blood cells in the blood, it could thus imply that though the extract may stimulate the production of red blood cells and hemoglobin, it could have an inhibitory effect on hemoglobin incorporation into red blood cells and a consequent reduction in oxygen exchange [46].

As [47] laments, immunomodulation through stimulation or suppression may help in maintaining a disease free state. Agents that activate host defence mechanisms in the presence of an impaired immune responsiveness can provide supportive therapy to conventional chemotherapy.

The significant increase in white blood cell and the differential leukocytes counts in the test animals shows that the methanolic extracts of *V. lasiopus* (O. Hoffman) may have immune boosting properties similar to those reported for garlic *Allum sativum* (Linn) by [36] and seed extracts of Citrus paradise Macfad by [48]. It has been reported that granulocyte-macrophage colony stimulating factor, macrophage colony stimulating factor, interleukins IL -2 IL-4 and IL-5 regulate the proliferation, differentiation and maturation of committed stem cells responsible for the production of white blood cells [49,50]. Since the methanolic extracts of *V. lasiopus* (O. Hoffman) caused increases in white blood cell counts, it is possible that the phytocompounds in the extracts stimulated the production of these regulatory factors or increased the sensitivity of the committed stem cells, responsible for the production of white blood cells (to the production of these factors.

Similar previous studies have shown the capacity of medicinal plants to induce immunostimulatory effects in animals. For instance, in a bid to prove the rasayana concept of Ayurveda, [51] demonstrated that Asparagus racemosus, Tinospora cordifolia (Willd) and Withania *somnifera* (Linn) protected animals against infections in normal and immunosuppressed states induced by hemisplenectomy or surgery. Furthermore, it has been shown these plants also produced leucocytosis and prevented the leucopenia induced by cyclophosphomide. The mode of action was found to be activation of the polymorphonuclear and monocyte-macrophage systems [51].

In their studies of root suspension of *Janakia arayalpathra*, [52] discovered the immunostimulatory properties of the suspension in mice. The suspension specifically stimulated an increase in humoral antibody titres and of antibody secreting spleen cells in the plaque forming cells assay following immunization with sheep erythrocytes. Moreover, it increased the number of peritoneal macrophages and induced an elevation of delayed hypersensitivity reaction in mice.

These stimulant effects could be associated with the adjuvant activity of some phytochemicals found in the extract. Saponins, alkaloids, tannins, phenolic compounds and flavonoids have generally been reported as immunostimulants [53,54]. That the methanolic extracts of *V. lasiopus* (O. Hoffman) elevated neutrophil counts in normal mice suggest that the extracts could help to increase the immunity against microbial infections [55].

The significant increase in platelets and their related parameter profiles after oral administration of methanolic extracts of *V. lasiopus* (O. Hoffman) suggests that the extracts contain compounds and phytochemicals that may have stimulated thrombopoietic process in normal mice.

As [56] suggested, platelets are involved in maintenance of normal blood homeostasis in organisms. MPV indicates platelet functions that include the release of thromboxane A2, beta thromboglobulin, platelet aggregation and more importantly the expression of glycogen Ib and IIb/IIIa receptors. In addition, [57,58] stated that certain vascular risk factors which include hypercholesterolaemia and diabetes mellitus increase MPV levels.

The significant increase in platelet count after oral administration of extracts of *V. lasiopus* (O. Hoffman) may indicate that the extracts have the potential to be developed as plant based therapeutic agents for thrombocytopenia. This is in line with [59], who reported that leaf juice of *Carica papaya* (Linn) consumed during the course of dengue infection had the potential to induce platelet production.

Moreover the significant increase in platelets and MPV after oral administration of methanolic extracts of *V. lasiopus* (O. Hoffman) may have been attributed to presence of tannins which have been shown to confer anti-hemorrhagic properties in animals. This is consistence with the findings of [60] that the sap of *Musa paradisiacal* is used for the treatment of fresh wounds, cuts and insect bites.

#### Conclusions

In conclusion the present study showed that oral administration of methanolic leaf extract of *V. lasiopus* (O. Hoffman) in normal mice resulted to a significant improvement of erythrocytic parameter profiles. This may suggest that the plants possess erythropoietin promoting activity and phytochemicals that slow down the natural process of oxidative breakdown of erythrocyte hence have a promising role in treatment and/or prevention of anemia.

That the significant increase in total white blood cell and differential White blood cell counts in normal mice after oral administration of the extract shows that the plants may promote the immunePage 5 of 6

stimulatory activities hence can be pursued for their clinical relevance in management of immunity-dependent disorders.

That the significant increase in platelet and their related parameters in normal mice after oral administration of the extract shows that the extracts has the potential to stimulate thrombopoietin production and can thus be used to manage hemostatic capacity of blood since platelets in blood mediate clotting mechanisms.

Furthermore, classes of phytochemicals in methanolic extracts of *V. lasiopus* (O. Hoffman) have previously been reported to have antioxidant activity hence can contribute significantly in reducing advancing age induced oxidative stress in elderly people.

The present study, therefore, scientifically confirms and supports the traditional use of leaves of *V. lasiopus* (O. Hoffman) in enhancing hematological parameters and improving health. In this study, the null hypothesis is hence rejected.

#### References

- 1. Cheeke PR, Shull LR (1999) Natural toxicants in feeds and poisonous plants. AVI Publishing, New York.
- Austin CC, Perkins SL (2006) Parasites in a biodiversity hotspot: a survey of hematozoa and a molecular phylogenetic analysis of Plasmodium in New Guinea skinks. Journal of Parasitology 92: 770-777.
- Jagger JE, Bateman RM, Ellsworth ML, Ellis CG (2001) Role of erythrocyte in regulating local O<sub>2</sub> delivery mediated by hemoglobin oxygenation. American journal of physiology Heart and circulatory physiology 280: H2833-2839.
- Mosmann TR, Coffman RL (1989) Th1 and Th2 cells: Different patterns of lymphokines secretion lead to different functional properties. Annual Review of Immunology 7: 145-173.
- 5. Paul H (2005) Platelet function analysis. Blood Reviews 19: 111-123.
- Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ (2009) Effects of aqueous leaf extract from the leaves of Chrysocoma ciliate (L) on some biochemical parameters of Wistar rats. Africa Journal of Biotechnology 8: 1425-1430.
- Price EA, Schrier SL (2008) Anemia in the elderly: introduction. Seminars in Hematology 45: 207-209.
- 8. Spivak JL (2000) The blood in systemic disorders. Lancet 355: 1707-1712.
- Tan-Shalaby J, Tempero M (1995) Malignancies after liver transplantation: a comparative review. Seminars in Liver Disease 15: 156-164.
- Blann A (2014) Routine blood tests 4: functions and diseases of red and white blood cells. Nursing Times 110: 16-18.
- Itterbeek P, Vandenberghe P, Nevens F, Fevery J, Aerts R, et al. (2002) Aplastic anemia after transplantation for non-A, non-B, non-C fulminant hepatic failure: case report and review of literature. Transplant International 15: 117-123.
- Bodey GP, Bolivar R, Fainstein V (1882) Infectious complications in leukemia patients. Seminar of Hematology 19: 193-220.
- American Society of Clinical Oncology (1994) Recommendations for the use of hematopoietic colony-stimulating factors: evidence-based, clinical practice guidelines. Journal of Clinical Oncology 12: 2471-2508.
- 14. Kawaguch K, Matsubara K, Uchida Y, Miyata K, Haseqawa D, et al. (2014) Successful treatment with allogenic hematopoietic stem cell transplantation of a severe congenital neutropenia patient harboring a novel ELANE mutation. The Japanese journal of clinical hematology 55: 2294-2299.
- 15. Izak M, Bussel JB (2014) Management of thrombocytopenia. F1000Prime Rep. 2: 6:45.
- Bradbury C, Murray J (2013) Investigating an incidental finding of thrombocytopenia. British Medical Journal 346:f11
- Bolton-Maggs PH, Chalmer EA, Collins PW (2006) A review of inherited platelet disorders with guidelines for their management on behalf of the UKHCDO. British Journal of Hematology 135: 603-633.
- Konkle BA (2011) Acquired disorders of platelet function. Hematology Am Soc Hematol Educ Program 2011: 391-396.

- 19. Consensus Conference on Platelet Transfusion (1998) Synopsis of background paper and consensus statement. British journal of Hematology 101: 609-617.
- Cheng Y, Wong RSM, Soo YOY (2003) Initial treatment of immune thrombocytopenic purpura with high-dose dexamethasone. New England Journal of Medicine 349: 831-836.
- Moake JL, (2002) Thrombotic microangiopathies. New England Journal of Medicine 347: 589-600.
- 22. Imaga NOA (2010) The use of phytomedicines as effective therapeutic agents in sickle cell anemia. Scientific Research and Essays 5: 3803-3807.
- Sumit D, Ripunjoy B, Nishant N (2014) A review on immune modulatory effect of some traditional medicinal herbs. Journal of Pharmaceutical, Chemical and Biological Sciences 2: 33-42.
- Glover PE, Stewart J, Gwynne MD (1966) Maasai and Kipsigis notes on East Africa plants, Part III- Medicinal uses of plants. East African Agricultural and Forestry Journal 32: 200-207.
- 25. Demissew S, Asfaw N (1994) some useful indigenous labiates from Ethiopia. Lamiales Newslett (Kew) 3: 5-6.
- 26. Neuwinger HD (2000) African traditional medicine. A dictionary of plant use and applications, Stuttgart: Medpharm Scientific Publishers.
- Kareru PG, Gachanja AN, Keriko JM, Kenji GM (2008) Antimicrobial activity of some medicinal plants used by herbalists in eastern province, Kenya. The African Journal of Traditional, Complementary and Alternative medicines 5: 51-55.
- 28. Kokwaro JO (1976) Medicinal Plants of East Africa, Kenya Literature Bureau, Kenya.
- 29. Jain NC (1986) Schalm's Veterinary Hematology. (4th Edn), Lea and Febiger, Philadelphia.
- 30. Harbone JB (1998) Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hal Publishersl 3: 60-66.
- 31. Kotake CK (2000) Practical Pharmacognosy. Vallabh Prakashan 4: 107-111.
- 32. Rodrigues B, McNeill JH (1992) The diabetic heart: metabolic causes for the development of cardiomyopathy. Cardiovascular Research 26: 913-922.
- 33. Ohlsson A, SM Aher (2009) Early erythropoietin for preventing red blood cell transfusion in preterm and/or low birth weight infants. Journal of Dietary Supplements 6: 227-251.
- 34. Polenakovic M, Sikole A (1996) Is erythropoietin a survival factor for red blood cells? Journal of the American Society of Nephrology 7: 1178-1182.
- Oyedeji KO, Bolarinwa AF (2013) Effect of Corchorus olitorius Extract On Haematological and Plasma Biochemical Parameters in Male Albino Rats. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) 5: 68-71.
- 36. Iranloye BO (2002) Effect of chronic garlic feeding on some hematological parameters. African Journal of biomedical Research 5: 81-82.
- 37. Mansi K, Lahham, J (2008) Effects of pride of Artemisia sieberi Besser (A/ herba-alba) on heart rate and some hematological values in. normal and alloxan induced diabetic rats. Journal of *Basic* and Appliced Sciences 4: 57-62.
- Kuppast IJ, Vasudeva NP, Ravi MC, Biradar SS (2009) Studies on the hematological effect of the extracts of Cordia dichotoma Forst F Fruits. Research Journal of Agriculture Science and Forestry 1: 195-2004
- Okpuzor J, Ogbunugafor HA, Kareem GK (2009) Hepatoprotective and hematologic effects of fractions of Globimetula braunii (Engle) in normal albino rats. EXCIL Journal 8: 182-189.
- 40. Esenowo GJ, Sam SM, Bala DN, Ekpo BAJ, Edung EM (2010) Phytochemical

screening and the hematological effect of Peristrophe bicalyculata (RETZ) diet preparation in albino rats. *World Journal of Science, Technology* 2: 277-281.

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- 41. Okon JE, Esenowo GJ, Etim GJ, Umoh NS (2013) Phytochemical Screening and Hemopoetic Study of the Ethanolic Root Extract of Baphia nitida (Lodd) on Albino Rats. International Journal of Modern Biology and Medicines. 3: 60-68
- 42. Chris W, Jenmie S, Steren W, Manunya N, James D (2008) Dietary antioxidant protect hemotopoietic cells and improve animal survival after total body irradiation. Journal of Radials Research 169: 384 =-366.
- Grassmann J (2005) Terpenoids as plant antioxidants. Vitamin and Hormones 72: 505-535.
- Ren WZ, Qiao H, Wang L, Zhu L, Zhang (2003) Flavonoids: promising anticanecr agents. Medical Research Reviews 23: 519-534.
- Chahar MN, Sharma MP, Dobhal YC (2011) Flavonoids: a versatile source of anticancer drugs .Pharmacognosy Review 5: 1-12.
- 46. Adebayo JO, Adesokan AA, Olatunji LA, Burro DO, Soladoye AO (2005) Effect of Ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. Biochemistry 17: 45.
- 47. Dahanukar SA, Kulkarni RA, Rege NN (2000) Pharmacology of medicinal plants and natural products. Indian Journal of Pharmacology 32: 81-118.
- Adeneye AA (2008) Hematopoietic effect of methanol seed extract of Citrus paradise Mac fad (grape fruit) in Westar rats. Biomedical Research 19: 23-26.
- Guyton AC, JE Hall (2000) A Textbook of Medical Physiology. (10th Edn), W.B. Saunders Co., Philadelphia.
- Ganong WF (2001) Review of Medical Physiology. (20th Edn), Lange Medical Books, McGraw Hill Companies Inc., New York.
- 51. Dahanukar SA, Thatte UM (1994) Current status of Ayurveda in phytomedicine. Phytomedicine. 359-368.
- 52. Subramoniam A, Rajasekharan S, Latha PG (1960)Immunomodulatory and antitumor activities of J. arayalpathra root. Fitoterapia 67: 140-144.
- Lakshmi V, Pandey K, Puri A, Saxena RP, Saxena KC (2003) Immunostimulant principles from Curculigo orchioides. Journal of Ethnopharmacology 89: 181-184.
- Dashputre NL, Naikwade NS (2010) Immunomodulatory Activity of Abutilon Indicum (linn) on Albino Mice. International Journal of Pharmaceutical Sciences and Research (IJPSR) 1: 178-184.
- 55. Gautam M, Diwanay S, Gairola S, Shinde Y, Patki P, et al. (2004) Immunoadjubent potential of Asparagus racemosus aqueous extract in experimental system. Journal of Ethnopharmacology 91: 251-255.
- Sharp DB, Bath PM, Martin JF (1995) Cigarette smoking sensitizes and desensitizes impedance-measured ADP-induced platelet aggregation in whole blood. Thrombosis and Haemostasis 74: 730-735.
- 57. Threatte GA (1993) Usefulness of the mean platelet volume. Clinical Laboratory Medicine 13: 937-950.
- Bath PM, Butterworth RJ (1996) Platelet size: measurement, physiology and vascular disease. Blood Coagulation and Fibrinolysis 7: 152-161.
- 59. Subenthiran S, Choon TC, Thayan R, Teck MK, Muniandy PK, et al. (2013) Carica papaya leaves juice significantly accelerates the rate of increase in platelet count among patients with dengue fever and dengue hemorrhagic fever. Evidence -Based Complementary and Alternative Medicine 1-7.
- Asquith TN, LG Butler (1986) Interaction of condensed Tannins with selected proteins. Photochemistry 25: 1591-1593.