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Effects of Methanol Seed Extract of *Aframomum melegueta* (Alligator Pepper) on Wistar Rats with 2,4-Dinitrophenylhydrazine-induced Hemolytic Anemia

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Effects of Methanol Seed Extract of *Aframomum melegueta* (Alligator Pepper) on Wistar Rats with 2,4-Dinitrophenylhydrazine-induced Hemolytic Anemia

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Abstract
The prevalence of parasitic infections such as malaria, which leads to decrease in hematological indices, the major cause of anemia, constitutes a serious health challenge in many developing countries such as Nigeria. This study investigated the effect of methanol seed extract of *Aframomum melegueta* on selected hematological indices of 2,4-dinitrophenylhydrazine (2,4-DNPH)-induced anemic rats model. The toxicity study and qualitative phytochemical screening of the extract were carried out using standard procedure. Twenty Wistar rats were grouped into five of four rats each ($n = 4$). Group I: Normal control; Group II: Negative control; Group III: administered 20 ml/kg b.w. of Astifer (Standard drug); Group IV and V were administered 200 and 400 mg/kg b.w. of the extract, respectively. The animals of Groups II to V were induced with 2,4-dinitrophenylhydrazine (20 mg/kg b.w.) once daily for seven consecutive days; their blood samples were collected by ocular puncture into heparinized capillary tubes for hematological analysis and animals with packed cell volume (PCV) $\geq$ 30% reduction were considered anemic for the study. The result of the qualitative phytochemical analysis showed that the methanol extract tested positive to alkaloids, carbohydrate, saponins, flavonoids, steroids, terpenoids, and anthraquinones. Acute toxicity and lethality studies on methanol extract showed an oral LD_{50} equal or less than 5000 mg/kg b.w. in mice. The rats administered 20 ml/kg b.w. of Astifer showed significant ($P < 0.05$) increase in PCV, hemoglobin, red blood cell (RBC) count, white blood cell (WBC) count, and neutrophils count compared with the normal control animals. The rats administered 200 mg/kg b.w. of *A. melegueta* showed significantly ($P < 0.05$) higher PCV and WBC count; non significant ($P > 0.05$) decrease in hemoglobin count, RBC, platelet, neutrophils and lymphocyte count compared with the normal control animals. The rats administered 400 mg/kg b.w. of *A. melegueta* showed significant ($P < 0.05$) increase in hemoglobin, platelet, erythrocyte sedimentation rate (ESR) and neutrophils count; non significant ($P > 0.05$) lower PCV, RBC, WBC count, and lymphocyte count compared with anemic rats administered with 0.3 ml of normal saline. It can be concluded that *Aframomum melegueta* seed has beneficial immunological and hematological properties in Wistar rats and possessed erythropoietic potentials at minimal dose that support its use for treating anemia.

Keywords: Anemia; Hematology; Dinitrophenylhydrazine; Astifer; Phytochemical.

1. INTRODUCTION

Anemia is one of the most common and widespread nutritional disorders in the world [1]. It is a common disorder of the blood, affecting about a quarter of the people globally [2]. Anemia contributed to the serious health problem in many malaria-endemic countries including Nigeria because malaria and other parasitic infections possibly lead to decrease in hemoglobin concentration.

Plants and their derivatives play key roles in world health and have long been known to possess biological activity [3]. Plants have been used to treat infectious diseases long before the discovery of microbes. A medicinal plant is any plant one or more of the parts of which contain bioactive substances that can be used for therapeutic purpose and are precursors for the discovery of drugs. Plants have been used in traditional medicine for many years due to their antimicrobial activity [4]. Alligator pepper (*Aframomum melegueta*) is a spice that is widely used in many cultures for entertainment, food flavor, and as part of many herbal medications [5]. Alligator pepper (*Aframomum melegueta*) is a very popular spice used mainly as food, in brewing, and in veterinary and traditional medicine [6]. It is generally assumed that the phytochemical compounds of medicinal plants contribute to their pharmacological efficacy. This present study evaluates the hematinic potential of *Aframomum melegueta* in 2,4-dinitrophenylhydrazine induced rats in search of anti-anemic herb.

2. MATERIALS AND METHODS

2.1. Plant Material

The seeds for this study (*Aframomum melegueta*) were obtained from Eke market, Afikpo, Ebonyi state, Nigeria. The seed was properly identified by the appropriate identification key, and the voucher specimen number of plant has been deposited in the herbarium at the Department of Botany, University of Nigeria, Nsukka. The outer coats of the seeds were removed, and the seeds were ground to powder, extracted with methanol, and dried to constant weight.

2.2. Extraction Procedure
Ground sample of *Aframomum melegueta* seeds was macerated with analytically graded methanol for 72 h with occasional string with a string rod. The extract was sieved with a sieving cloth; the filtrate was passed through filter paper, concentrated by rotary evaporator, and dried at room temperature.

2.3. Animals
Twenty Wistar albino rats used for this study were purchased from the Department of Veterinary Medicine, University of Nigeria, Enugu state. They were feed *ad libitum* with commercial feed and were allowed to acclimatize for seven days under standard laboratory conditions in a clean rat cage with four rats per cage in the Science Laboratory Technology animal house of Akanu Ibiam Federal Polytechnic, Nigeria.

2.4. Experimental Design
The animals were grouped into five with four animals per group ($n = 4$).
Group I: Nonanemic rats administered 0.3 ml of normal saline.
Group II: 2,4-dinitrophenylhydrazine-induced anemic rats administered 0.3 ml of normal saline.
Group III: 2,4-dinitrophenylhydrazine-induced anemic rats treated with 20 ml/kg body weight (b.w.) multivitamin (astifer).
Group IV: 2,4-dinitrophenylhydrazine-induced anemic rats treated with 200 mg/kg b.w. methanol extract of *A. melegueta* seed.
Group V: 2,4-dinitrophenylhydrazine-induced anemic rats treated with 400 mg/kg b.w. methanol extract of A. *melegueta* seed.

2.5. Induction of Hemolytic Anemia with 2,4-Dinitrophenylhydrazine
A modified method described by Berger [7] was used in this study. The animals of Groups II to V received 2,4-dinitrophenylhydrazine (20 mg/kg body weight) once daily for seven days. On the eighth day, their blood samples were collected by ocular puncture of each rat for hematological analysis. Rats with ≥30% reduction in packed cell volume (PCV) were considered anemic and used for this study.

2.6. Drug Treatment
The anemic Group IV and V received 200 and 400 mg/kg b.w. of the extract, respectively, and Group III received standard hematinic drug astifer (20 ml/kg b.w.). All drugs were administered once daily for 10 consecutive days by oral-feeding cannula.

2.7. Acute Toxicity and Lethality (LD_{50}) Test
The acute toxicity study of ethanol extract of *Aframomum melegueta* seed was carried out using the modified Lorke [8] method. The test was divided into two phases. In the first phase, total of nine randomly selected adult mice were divided into three groups ($n = 3$) and received 10,100 and 1000 mg/kg b.w. of the extract, and the signs of toxicity and number of death were recorded after 24 hours of observation. The doses for phase two were determined based on the outcome of the phase one. Since there was no death recorded, a fresh batch of animals were used following the same procedure with higher doses of 1900, 2600, and 5000 mg/kg body weight of the extract. The animals were also observed for 24 hours for signs of toxicity and death. The LD_{50} was calculated as the geometric mean of the high nonlethal dose and lowest lethal dose [8].

2.8. Phytochemical Screening
Basic qualitative phytochemical screening of methanol seed extract of *Aframomum melegueta* seed was carried out by testing the presence or absence of the following plant constituents: flavonoids, tannins, saponins, alkaloids, carbohydrates, trepenoids, anthraquinones steroids, and phenol using the methods outline by Trease and Evans [9].

2.9. Hematological Analysis
The animals were anesthetized and blood samples were obtained through ocular puncture into ethylenediaminetetraacetic acid (EDTA) bottles using heparinized capillary tubes. The animals were sacrificed, and more blood samples were obtained through cardiac puncture into their respective EDTA bottles. The hemoglobin (HB) concentration, PCV, red blood cell (RBC) count, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), neutrophil count, and platelet count were determined according to the method outlined by Dacie and Lewis [10].

2.10. Data Analysis
The data obtained were analyzed using analysis of variance (ANOVA). The data were further subjected to LSD *post hoc* test for multiple comparisons and differences between means regarded significant at $P < 0.05$. The results were expressed as mean ± SEM.

3. RESULTS

3.1. Extract Yield of the Methanol Seed Extract of *Aframomum melegueta*
The yield of the extract was observed to be 3.10 g (2.60%).

Table 1. Qualitative phytochemical screening of methanol seed extract of *A. melegueta*.

Phytochemical constituents	Methanol extract
Alkaloids	+
Carbohydrates	+
Saponins	++
Flavonoids	+
Steroids	+++
Tannins	ND
Trepenoids	++
Anthraquinones	+
Phenols	ND

KEY: ND: Not detected; +: Low; ++: Moderate; +++: High

3.2. Lethal Dose (LD_{50})

In the investigation, there was no lethality or behavioral changes in the three groups of mice that received 10, 100, and 1000 mg/kg b.w. of the extract at the end of first phase of the experiment. Based on this result, further increased doses of 1900, 2600, and 500 mg/kg b.w. of the extract were administered. There was neither death nor behavioral change in the animals that received 1900 and 2600 mg/kg b.w. Those that received 5000 mg/kg body weight of the extract showed weakness, but no death was observed within 24 hours of observation. This result shows that the methanol seed extract of *A. melegueta* is safe at doses below 5000 mg/kg.

3.3. Phytochemical Screening of Methanol Seed Extract of *Aframomum melegueta*

The qualitative phytochemical screening as observed in Table 1 showed relatively high presence of steroids, moderate presence of bioactive compounds such as saponnins and terpeniods. Alkaloids, carbohydrates, flavonoids, and anthraquinones were present in relatively low concentration, while tannins and phenols were not detected in the sample.

3.4. Effect of Methanol Seed Extract of *A. melegueta* on Packed Cell Volume and Hemoglobin Count of 2,4-Dinitrophenylhydrazine-induced Anemic Rats

As illustrated in Figure 1, the rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant ($P < 0.05$) decrease in PCV compared with the normal control animals. The rats administered 20 ml/kg b.w. of astifer showed significant ($P < 0.05$) increase in PCV compared with anemic rats administered 0.3 ml of normal saline. The rats administered 200 mg/kg b.w. of *A. melegueta* extract showed significant ($P < 0.05$) increase in PCV compared with anemic rats administered 0.3 ml of normal saline. There was significant ($P < 0.05$) decrease in the PCV of animals induced with 2,4-DNPH treated with high dose of the extract compared with the induced rats administered 200 mg/kg b.w. of the extract.

The anemic rats administered 20 ml/kg b.w. of astifer showed significant ($P < 0.05$) increase in hemoglobin count compared with the normal control rats and anemic rats administered 0.3 ml of normal saline. The rats administered various doses of the extract showed significant ($P < 0.05$) decrease in hemoglobin count compared with 2,4-DNPH-induced anemic rats administered 20 ml/kg b.w. of astifer. The anemic rats treated with 400 mg/kg b.w. of the extract showed significantly ($P < 0.05$) higher hemoglobin count compared with the anemic rats administered 200 mg/kg b.w. of the extract.

3.5. Effect of Methanol Seed Extract of *A. melegueta* on Red Blood Cell Count (RBC) and Platelet Count of 2,4-Dinitrophenylhydrazine-induced Anemic Rats

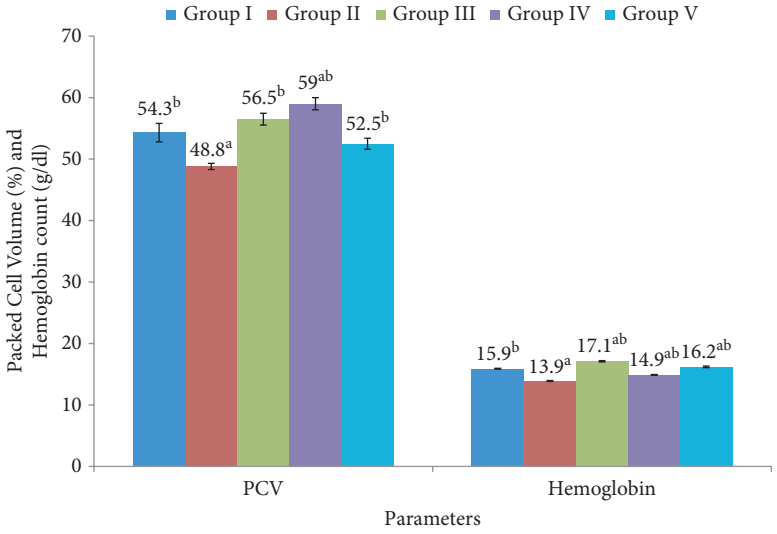
As illustrated in Figure 2, the rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant ($P < 0.05$) decrease in RBC compared with the normal control animals. The rats administered 20 ml/kg b.w. of astifer showed significant ($P < 0.05$) increase in RBC compared with animals in Groups I and II. The rats administered various doses of the extract showed significantly ($P < 0.05$) higher RBC count compared with 2,4-DNPH-induced rats administered 20 ml/kg b.w. of astifer.

The rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant ($P < 0.05$) decrease in platelet count compared with the normal control animals. The anemic rats administered 20 ml/kg b.w. of astifer showed significant ($P < 0.05$) increase in platelet count compared with group II animals. The rats administered various doses of the extract showed significant ($P < 0.05$) increase in platelet count compared with 2,4-DNPH-induced anemic rats administered 20 ml/kg b.w. of astifer.

3.6. Effect of Methanolic Seed Extract of Aligator Pepper (*A. melegueta*) on White Blood Cell Count (WBC) and Erythrocyte Sedimentation Rate (ESR) of 2,4-Dinitrophenylhydrazine-induced Anemic Rats

As illustrated in Figure 3, the rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant ($P < 0.05$) decrease in WBC compared with the normal control animals. The rats administered 20 ml/kg b.w. of astifer showed significant

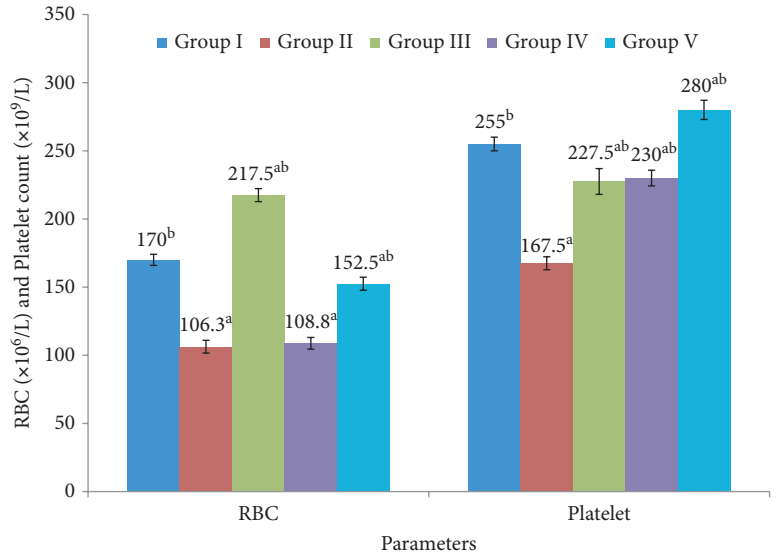
Figure 1: Packed cell volume (PCV) and hemoglobin count of 2,4-dinitrophenylhydrazine induced anemic rats treated with methanolic extract of *Aframomum melegueta*.



$^{a}P < 0.05$: Significant compared with Group I. $^{b}P < 0.05$: Significant compared with Group II.

Group I: Normal control rats
Group II: 2,4-Dinitrophenylhydrazine-induced rats administered 0.3 ml of normal saline
Group III: 2,4-Dinitrophenylhydrazine-induced rats administered 20 ml/kg b.w. of astifer
Group IV: 2,4-Dinitrophenylhydrazine-induced rats administered 200 mg/kg b.w. of *A. melegueta* extract
Group V: 2,4-Dinitrophenylhydrazine-induced rats administered 400 mg/kg b.w. of *A. melegueta* extract

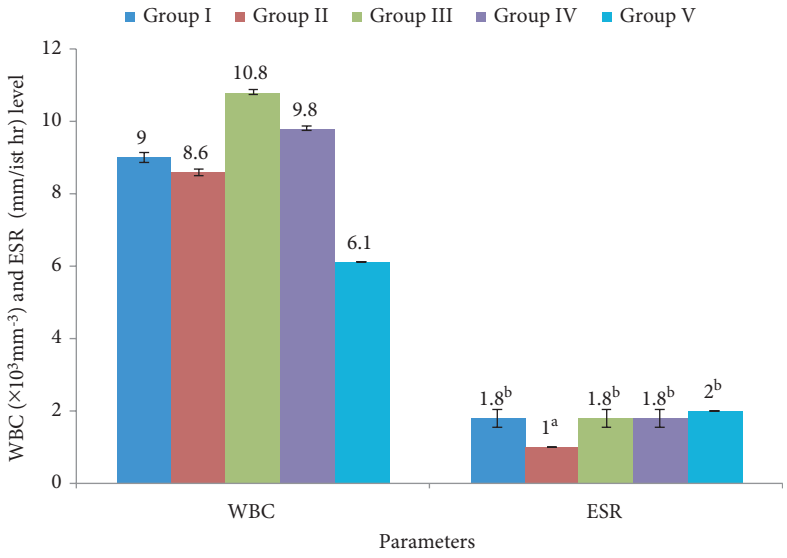
Figure 2: Red blood cell count (RBC) and platelet count of 2,4-dinitrophenylhydrazine-induced anemic rats treated with methanolic extract of *Aframomum melegueta*.



$^{a}P < 0.05$: Significant compared with Group I. $^{b}P < 0.05$: Significant compared with Group II.

Group I: Normal control rats
Group II: 2,4-Dinitrophenylhydrazine-induced rats administered 0.3 ml of normal saline
Group III: 2,4-Dinitrophenylhydrazine-induced rats administered 20 ml/kg b.w. of astifer
Group IV: 2,4-Dinitrophenylhydrazine-induced rats administered 200 mg/kg b.w. of *A. melegueta* extract
Group V: 2,4-Dinitrophenylhydrazine-induced rats administered 400 mg/kg b.w. of *A. melegueta* extract

Figure 3: White blood cell count (WBC) and erythrocyte sedimentation rate (ESR) of 2,4-dinitrophenylhydrazine-induced anemic rats treated with methanolic extract of *Aframemum melegueta*.



[a]P < 0.05: Significant compared with Group I. [b]P < 0.05: Significant compared with Group II.

Group I: Normal control rats
Group II: 2,4-Dinitrophenylhydrazine-induced rats administered 0.3 ml of normal saline
Group III: 2,4-Dinitrophenylhydrazine-induced rats administered 20 ml/kg b.w. of astifer
Group IV: 2,4-Dinitrophenylhydrazine-induced rats administered 200 mg/kg b.w. of *A. melegueta* extract
Group V: 2,4-Dinitrophenylhydrazine-induced rats administered 400 mg/kg b.w. of *A. melegueta* extract

(P < 0.05) increase in WBC compared with animals in Groups I and II. The rats administered various doses of the extract significantly (P < 0.05) lowered the WBC compared with 2,4-DNPH-induced rats administered 20 ml/kg b.w. of astifer.

The rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant (P < 0.05) decrease in ESR compared with the normal control animals. The anemic rats administered 20 ml/kg b.w. of astifer showed significant (P < 0.05) increase in ESR compared with Group II animals. The anemic-induced rats treated with 400 mg/kg b.w. of the extract significantly (P < 0.05) increase ESR compared with 2,4-DNPH-induced anemic rats administered 20 ml/kg b.w. of astifer.

3.7. Effect of Methanol Seed Extract of *A. melegueta* on Neutrophils and Lymphocytes Count of 2,4-Dinitrophenylhydrazine-induced Anemic Rats

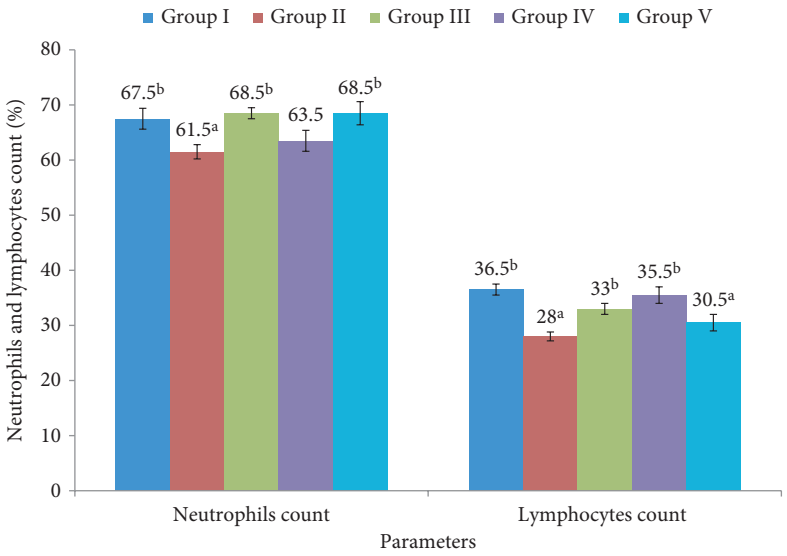
As illustrated in Figure 4, the rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant (P < 0.05) decrease in neutrophils count compared with the normal control animals. The rats administered 20 ml/kg b.w. of astifer showed significant (P < 0.05) increase in neutrophils count compared with animals in Groups I and II. The anemic rats treated with 200 mg/kg b.w. of the extract significantly (P < 0.05) decrease in neutrophils count compared with 2,4-DNPH-induced rats administered 20 ml/kg b.w. of astifer, while the anemic rats administered 400 mg/kg b.w. of the extract showed significantly (P < 0.05) higher neutrophils count compared with 2,4-DNPH-induced rats treated with 200 mg/kg b.w. of the extract.

The rats induced with 2,4-DNPH administered 0.3 ml of normal saline showed significant (P < 0.05) decrease in lymphocytes count compared with the normal control animals. The anemic rats administered 20 ml/kg b.w. of astifer showed significant (P < 0.05) increase in lymphocytes count compared with Group II animals. The anemic rats treated with 200 mg/kg b.w. of the extract showed significantly (P < 0.05) higher lymphocytes count compared with 2,4-DNPH-induced anemic rats administered 20 ml/kg b.w. of astifer, while the anemic rats administered 400 mg/kg b.w. of the extract significantly (P < 0.05) lowered lymphocyte count compared with 2,4-DNPH-induced anemic rats administered 20 ml/kg b.w. of astifer and 200 mg/kg b.w. of the methanol seed extract.

4. DISCUSSION

Many natural medicinal products have been used for the treatment of multiple ailments [11]. Although many have been displaced by conventional pharmaceutical approaches; there is currently a renewal of interest in the use of natural products by the public [12]. The hematological parameters of 2,4-DNPH-induced anemic rats treated with astifer and methanol seed extract of

Figure 4: Neutrophils and lymphocytes count of 2,4-dinitrophenylhydrazine-induced anemic rats treated with methanolic extract of *Aframomum melegueta*.



$^{a}P < 0.05$: Significant compared with Group I. $^{b}P < 0.05$: Significant compared with Group II.

Group I: Normal control rats
Group II: 2,4-Dinitrophenylhydrazine-induced rats administered 0.3 ml of normal saline
Group III: 2,4-Dinitrophenylhydrazine-induced rats administered 20 ml/kg b.w. of astifer
Group IV: 2,4-Dinitrophenylhydrazine-induced rats administered 200 mg/kg b.w. of *A. melegueta* extract
Group V: 2,4-Dinitrophenylhydrazine-induced rats administered 400 mg/kg b.w. of *A. melegueta* extract

A. melegueta indicated that the mean PCV, hemoglobin concentration, RBC count, WBC count, and neutrophils count increased significantly ($P < 0.05$), while the platelet and lymphocytes concentration were observed to be significant ($P < 0.05$) reduction compared with normal control rats. As observed in this study, the standard drug (astifer) had some positive effect on the production of blood cells in the tested rats; this was observed by the increase in PCV, HB, RBC, WBC, and neutrophils concentration of anemic rats after the administration of the standard drug at 20 ml/kg b.w. and the increase in WBC could be due to the defense mechanism against the entrance of foreign material in the body system of the rats [13]. The increase in PCV is an indication of increase hemoglobin concentration that may result from increased RBC count [14]. WBCs form part of the immune system in animals working against invading pathogens; the significant ($P < 0.05$) increase in WBC counts of the dinitrophenylhydrazine-treated animals when compared with the control animals is due to the ability of DNPH to act as hapten, thereby stimulating the production of plasma-cell derivatives of β-cells [15].

The mean PCV and WBC count, increased significantly ($P < 0.05$) at 200 mg/kg b.w. of *A. meleguta* extract administration, while the hemoglobin count, RBC count, platelet, neutrophils, and lymphocyte decreased significantly ($P > 0.05$) at the same concentration of the methanol seed extract compared with the untreated rats; methanol seed extract of *A. melegueta* must have influenced the defense mechanism and immunity of the tested rats. The mean hemoglobin, platelet, ESR, and neutrophils count concentration increased significantly ($P < 0.05$) at 400 mg/kg b.w. of *A. melegueta* extract administration, while the PCV, RBC count, WBC count, and lymphocyte count concentration decreased significantly ($P > 0.05$) at the same administration of the extract compared with the control animals.

Hemoglobin is a natural constituent of RBCs and biochemically adapted to carry oxygen in the lungs and deposit it at tissues for oxidative metabolism [15]; it has been characterized to also play a major role in physiological carbon dioxide removal and acid–base balance; an increased production of hemoglobin is an advantage to an organism [15]. This metabolic status can only be ensured by decrease in RBC destruction or increased RBC production [16]. The increase in hematological parameters investigated could be as a result of some constituents such as iron and some B-complex vitamin that the plant extract possess as these serves as hematopoietic factors that influence directly blood cells production in the bone marrow [13, 17]. The results of this study were consistent with the observations of the authors who had reported significant increase in hematological parameters of 2,4-dinitrophenylhydrazine-induced anemic rats treated with various natural products such as fluted pumpkin [13, 18]. The continuous exposure of the body systems of the rats to the medicinal products may cause lymphocytosis that may account for the use of this plant for medicinal purposes. The result of the qualitative phytochemical analysis observed in this study indicated that the constituents alkaloids, carbohydrates, saponins, trepenoids, and anthraquinones were present in the seed. The bioactive compounds detected such as flavonoids have been implicated in the restoration of blood level in the body [19].

5. CONCLUSION

The methanol seed extract of *A. melegueta* has erythropoietic potential and immunological properties at different doses used in this study and may be included in the management of life-threatening ailments including anemia and diverse infections that can result in anemia.

Author Contributions

This study was carried out with contribution from all the authors. Authors DAO and FE designed the study, wrote the protocol, and supervised the work. Authors DAO, FE and AOA carried out all laboratory work. Author DAO performed the statistical analysis. Authors DAO and KCN managed the analyses of the study. Authors DAO, FE, and AOA wrote the first draft of the manuscript. Authors DAO, KCN, and FE managed the literature searches, and all authors read, edited, and approved the final draft of the manuscript.

Source of Funding

None.

Conflict of Interest

No conflict of interest is associated with this work.

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