# *Original Research Article*

## EFFECTS OF ANTI-SICKLING POLYHERBAL MIXTURE ON HAEMATOLOGICAL INDICES IN NORMAL AND ANAEMIA-INDUCED RABBITS


### Abstract

Some plants used in the management of SCD have been shown to increase gelling time of sickle cell blood and inhibits sickling in vitro, reversal of sickling, inhibiting osmotically induced haemolysis of erythrocytes, membrane stabilization. Some plants such as *Sorghum bicolar, Phyllanthus amarus, U. afzelii*, *Securidaca longipedunculata*, *Momordica charantia*, *Dalium guineense* have been found to exhibit anti-sickling properties. The polyherbal combination of these drugs was used in this study for the investigation of the effects of anti-sickling polyherbal mixture on haematological indices in rabbits. Sixty (60) New Zealand rabbits weighing 1200g ±200g, conducted in duplicate and designated experiment X (normal rabbits) and Y (anaemia induced using Cadmium 2mg/kg +10 mg/Kg body weight phenylhydrazine for 15 days). Both groups were further subdivided into four groups (A-D) with 5 animals each, administered saline substitute, different grades of polyherbal mixtures for 8 weeks. Blood sample (2mls) was collected into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for full blood count. The results in the experiment (X) showed significant increase in WBC ($10^3$/µl) count across the groups (B=8.15±0.33; C=9.3±0.74; D=9.8±0.59) compared to the control group (A=7.25±0.44) ($p<0.05$). RBC ($10^6$/µl) count showed insignificant increase in group B (5.1±0.20) and C (5.6±0.450), decrease level in D (4.4±0.26) compared to control A (4.8±0.29) ($p<0.05$). Hb(g/dl) and HCT (%) showed similar pattern with insignificant increased levels across all treatment groups (B=14.8±0.59, 43.4±1.74; C=15.5±0.78, 45.8±3.; D=14.2±0.85, 43.7±2.62) compared to control A (13.6±0.68; 41.5±2.49) respectively. Platelets showed significant increase in group B and C (286±17.16 and 286±17.16) compared to control A (244±9.76) with significant decrease in group D (226±18.08) ($p<0.05$). In experiment (Y), WBC count showed significant increase across all treatment groups (B= 6.4±0.26 C= 6.8±0.54 D =10.6±0.64) compared to control (A= 4±0.24) ($p<0.05$). RBC ($10^6$/µl) count in control A gave (2.5±0.15), insignificant increase in B (3.1±0.12) with significant increase in group C (3.5±0.28) and insignificant decrease in D (2.2±0.13) ($p<0.05$). Higher level of Hb (g/dL) was seen in group B and C (B= 8.4±0.34; C= 9.1±0.46), while D (7.7±0.46) had a slightly elevated value compared to the control A (7.1±0.36) ($p<0.05$). HCT (%) showed significant increase across the groups (B=26±1.04; C=28±2.24; D=24±1.44) ($p<0.05$) compared to control A (18±1.08). Red cell indices showed some degree of derangement across the study groups. Findings in this study suggest that polyherbal mixture have a positive effect on the haemoglobin, red blood cells, packed cell volume and white blood cell count of the rabbits in a dose dependent manner.



**Keywords**: Sickle cell disease (SCD), Anti-Sickling, Polyherbal-Mixture, Haematological Indices, Anaemia-induced.


## INTRODUCTION

The discovery of natural plant extracts and products have been used in the formulation of drugs for the management of various diseases in West Africa where sickle cell anaemia (SCA) is endemic. Plant extracts have been found to possess anti-sickling properties which prevent erythrocytes from deforming and losing its integrity. Some plants used in the management of SCD have been shown to increase gelling time of sickle cell blood and inhibits sickling in vitro, reversal of sickling, inhibiting osmotically induced haemolysis of erythrocytes, membrane stabilization.


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Sickle cell disease (SCD) is an autosomal recessive hereditary blood disease/disorder that is passed down from parents to offspring. It is a serious disorder in which the body makes red blood cells that are sickle-shaped (1,2). SCD widely has no cure and the health-care cost of the management of SCD patients is disproportionately high compared to the number of people afflicted by the disease. The individuals affected by this disease are mostly below poverty line and unable to afford the high-cost of treatment. Due to the debilitating effect and cost of managing the SCD, research has been going on to determine the efficacy of certain natural and artificial agents having anti-sickling effect, aiming at having a suitable and permanent cure for this health problem (3). The management of sickle cell anaemia is usually expensive and often not affordable by the poor. Most drugs that are available for the management of SCD are insufficiently effective, too expensive and toxic (4).

Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (5,6) and are of ecological and physiological interest in aiding the understanding of the relationship of blood characteristics to the environment (7). Haematological parameters are measurable index of the haematopoietic (blood) system used to access the normality, functionality of the blood of an individual for the purpose of diagnosis vis a vis establishing a state of health or disorder (5,6). SCD has been shown to affect various cell lineages. The production of mature blood cell, which could be a red blood cell, white blood cell, or some other type of blood cell is referred to as haematopoiesis (8).

The discovery of natural plant extracts and products have been used in the formulation of drugs for the management of various diseases in West Africa where sickle cell anaemia (SCA) is endemic (9). About 75% of all SCD patients live in sub-Saharan Africa. Annually, Nigeria records the highest incidence of this disorder worldwide, with ~91 011 birth defects and about 100,000 infant deaths (10). Different natural products have been used in SCD patients such as vegetal food/fruits rich in dietary supplement, food with assorted herbs/spices, treatment for painful crisis using analgesics and plenty of fluids (9). Many of such drugs have been discovered and used for the management of sickle cell disease. However, there is no promising drug for the treatment of sickle cell disease yet.

Plant extracts have been found to possess anti-sickling properties which prevent erythrocytes from deforming and losing its integrity (11). Some plants used in the management of SCD have

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been shown to increase gelling time of sickle cell blood and inhibits sickling in vitro, reversal of sickling, inhibiting osmotically induced haemolysis of erythrocytes, membrane stabilization (12). Recent therapy focuses on the erythrocytic rehydration management of sickle cell disease (SCD) hence, involves substances which has an ability to rehydrate the erythrocytes and furthermore, preventing it from losing its shape (11).

Phenylhydrazine (PHZ) is known to induce anaemia since decades (13). It is a haemolytic agent (14) and interacts with the membrane lipid of erythrocytes in oxidation reactions, which results in the generation of destructive free radicals, which are responsible for subsequent haemolysis and haemolytic anaemia. Hence its use in experimental studies using animal models (13). Some plants such as *Sorghum bicolar*, *Phyllanthusamarus* and others such as *U. afzelii*, *Securidacalongipedunculata*, *Momordicacharantia*, *Daliumguineense* have been found to exhibit anti-sickling properties (15,16,10), hence the need to further investigate their effect. The polyherbal mixture in this study is a combination of the above mentioned plants which contain phytochemicals (compounds produced by plants believed to protect cells from damage).

Following high incidence of cases of sickle cell disease without any known therapeutic drug for the management or treatment of the condition, paucity of information on the use and efficacy of anti-sickling polyherbal mixture on haematological indices in cases of sickle cell disease, this work was set up to investigate the effects of anti-sickling polyherbal mixture on haematological indices in rabbits.

## MATERIALS AND METHODS

### Animal Preparation

The Rabbits for this study were obtained from the Animal House of the College of Medicine and Health Sciences, Afe Babalola University. Ado-Ekiti. The Rabbits diets were prepared at ABUAD farm. Mineral and vitamin mixtures were prepared according to the recommendations for rabbits of the American Institute of Nutrition (17). The animals were housed under standard laboratory condition of good lighting, moderate temperature and adequate ventilation in a hygienic environment. This experiment was conducted in duplicate and designated experiment X and Y with each set with each consisting of 30 animals divided into 4 groups and given various treatments.

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**Experimental Design and Polyherbal Mixture Administration**

**Chart 1. EXPERIMENT X** consisted of twenty (20) male and female rabbits assigned into four groups of five (5) animals each consisting of three (3) males and two (2) females. They were acclimatized for a period of two (2) weeks, after which they were treated as follows:

| GROUPS | NUMBER OF RABBITS (n) | Polyherbal Mixture (mg/kg) |
|---|---|---|
| Group A | 5 | Saline substitute |
| Group B | 5 | 250 |
| Group C | 5 | 500 |
| Group D | 5 | 750 |

Chart 2. Experiment Y consisted of twenty (20) male and female rabbits assigned into four groups of five (5) animals each consisting of three (3) males and two (2) females. They were acclimatized for a period of two (2) weeks, after which they were treated as follows:

| GROUPS | NUMBER OF RABBITS (n) | Polyherbal Mixture (mg/kg) | Cadmium + Phyenylhydrazine |
|---|---|---|---|
| Group A | 5 | Saline substitute | Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days) |
| Group B | 5 | 250 | Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days) |
| Group C | 5 | 500 | Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days) |

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| | | | |
|---|---|---|---|
| Group D | 5 | 750 | Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days) |

**Collection of Blood Sample and Analysis**

Blood sample (2ml) was obtained from the marginal ear vein (18) of the Rabbits after the period of acclimatization (2 weeks) which will provide baseline data and after the treatment (8weeks) into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles for measurement of variables**.** Determination of full blood count was done as described by Barbara *et al.*, (19)

**Statistical Analysis**

Data obtained was analysed using the Statistical Package for Social Sciences (SPSS) version 21.0 software. Analysis of Variance (ANOVA) and Independent Student T-test as well as Post Hoc test were used to compare groups and results presented in bar charts.

**RESULTS**

The results in the normal rabbits experiment (X) showed statistically significant increase in WBC ($10^3$/µl) count across the groups (B=8.15±0.33; C=9.3±0.74; D=9.8±0.59) compared to the control group (A=7.25±0.44) ($p<0.05$). RBC ($10^6$/µl) count showed insignificant increase in group B (5.1±0.20) and C (5.6±0.450), decrease level in D (4.4±0.26) compared to control A (4.8±0.29) ($p<0.05$). Hb(g/dl) and HCT (%) showed similar pattern with statistically insignificant increased levels across all treatment groups (B=14.8±0.59, 43.4±1.74; C=15.5±0.78, 45.8±3.; D=14.2±0.85, 43.7±2.62) compared to control A (13.6±0.68; 41.5±2.49) respectively. Platelets showed statistically significant increase in group B and C (286±17.16 and 286±17.16) ($p<0.05$) compared to control A (244±9.76) with statistically significant decrease in group D (226±18.08) ($p<0.05$). MCV (fl) showed no statistically significant difference in control A (85±3.40) and treatment group B (85±3.45). However, a significant decrease in C (81±4.05) ($p<0.05$) and insignificant increase in D (99±5.94) were recorded. MCH (pg) showed insignificant increase in group B (29±1.16) and D (32±1.92) compared to control group A (28±1.68), with insignificant decrease in C (27±2.16). MCHC (g/dl) showed insignificant

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increase in group B (34±1.36) and C (35±2.80) compared to control A (33±1.98) with a statistically insignificant decrease in D (32±1.92).

In the anaemia induced experiment (Y), WBC count showed significant increase across all treatment groups (B= 6.4±0.26 C= 6.8±0.54 D =10.6±0.64) compared to control (A= 4±0.24) ($p<0.05$). RBC ($10^6$/µl) count in control A gave (2.5±0.15). Statistically insignificant increase was seen in B (3.1±0.12) while C (3.5±0.28) showed statistically significant increase ($p<0.05$) with statistically insignificant decrease in D (2.2±0.13). Higher level of Hb (g/dL) was seen in group B and C (B= 8.4±0.34 and C= 9.1±0.46), while D (7.7±0.46) had a slightly elevated value compared to the control A (7.1±0.36) ($p<0.05$). HCT (%) showed a statistically significanr67t increase across the groups (B=26±1.04; C=28±2.24 and D=24±1.44) ($p<0.05$) compared to control A (18±1.08). MCV (fl) showed significant increase in B (84±3.36) and C (79±3.95) compared to control group A (72±2.88) and statistically insignificant increase in D (98±5.88). MCH (pg) values for control group A (27±1.62) compared with B (29±1.16) showed insignificant increase, insignificant decrease in C (26±2.08) and significant increase in D (34±2.04) ($p<0.05$). MCHC (g/dL) showed no statistically significant difference in control group A (32±1.92) and D (32±1.92) with statistically significant increase in B **(**39±1.56) and C **(**33±2.64) ($p<0.05$) as shown in figure 1-10.

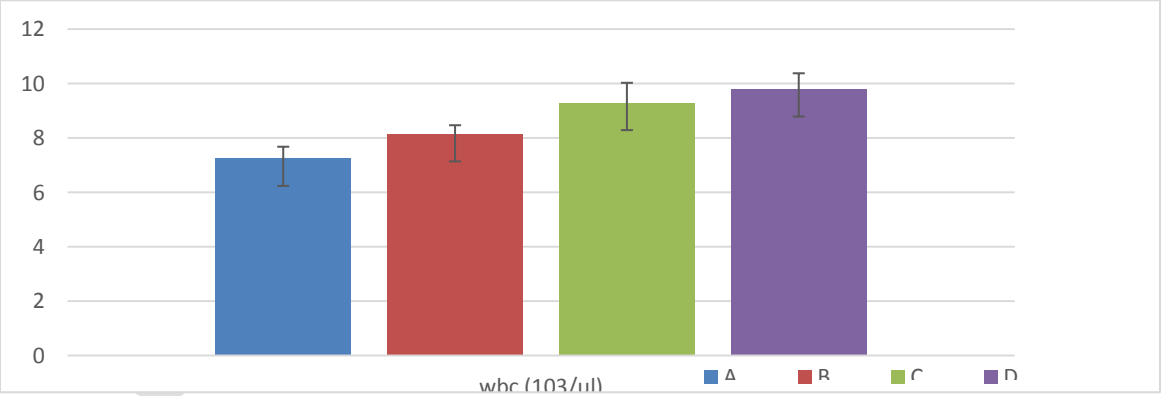


FIGURE 1: TOTAL WHITE BLOOD CELL COUNTS OF NORMAL RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A- (Saline substitute).

Group B- 250 mg/kg Polyherbal mixture.

Group C- 500 mg/kg Polyherbal mixture.

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Group D- 750 mg/kg Polyherbal mixture

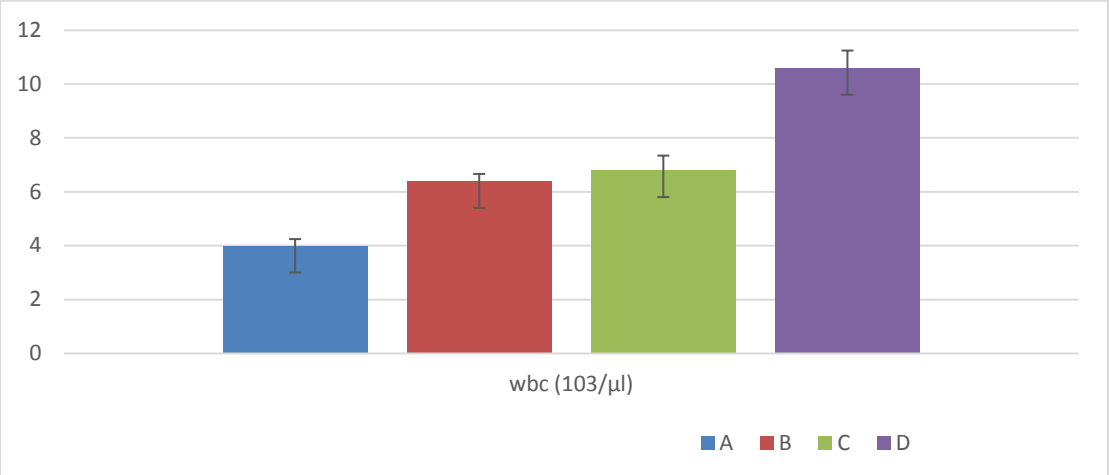


FIGURE 2: TOTAL WHITE BLOOD CELL COUNTS OF INDUCED RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days).

Group B - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days).

Group C - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

Group D - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

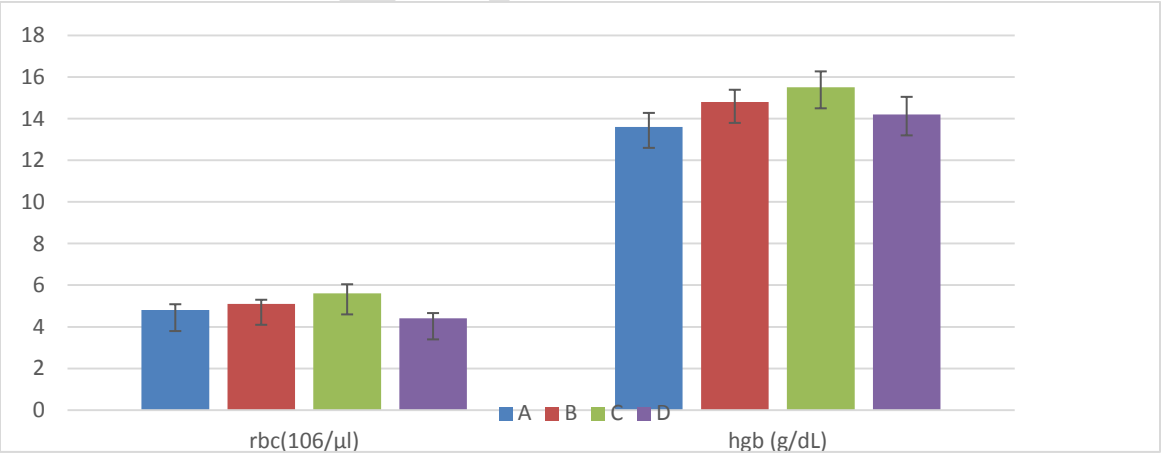


FIGURE 3: TOTAL RED BLOOD CELL COUNTS AND HAEMOGLOBIN OF NORMAL RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A- (Saline substitute)

Group B- 250 mg/kg Polyherbal mixture

Group C- 500 mg/kg Polyherbal mixture

Group D- 750 mg/kg Polyherbal mixture

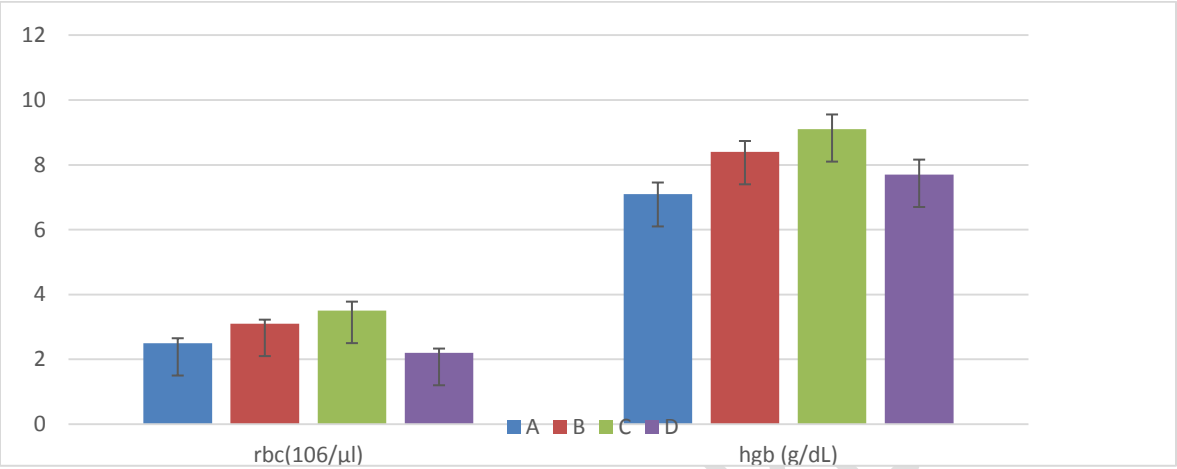


FIGURE 4: TOTAL RED BLOOD CELL COUNTS AND HAEMOGLOBIN OF INDUCED RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

Group B - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

Group C - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

Group D - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

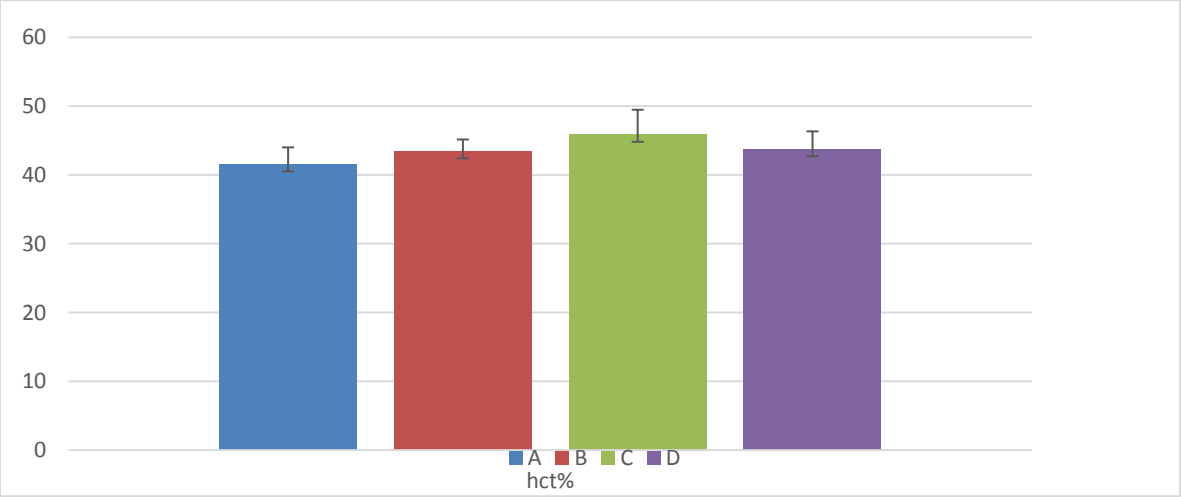

FIGURE 5: PERCENTAGE HAEMATOCRIT OF NORMAL RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A- (Saline substitute)
Group B- 250 mg/kg Polyherbal mixture
Group C- 500 mg/kg Polyherbal mixture
Group D- 750 mg/kg Polyherbal mixture

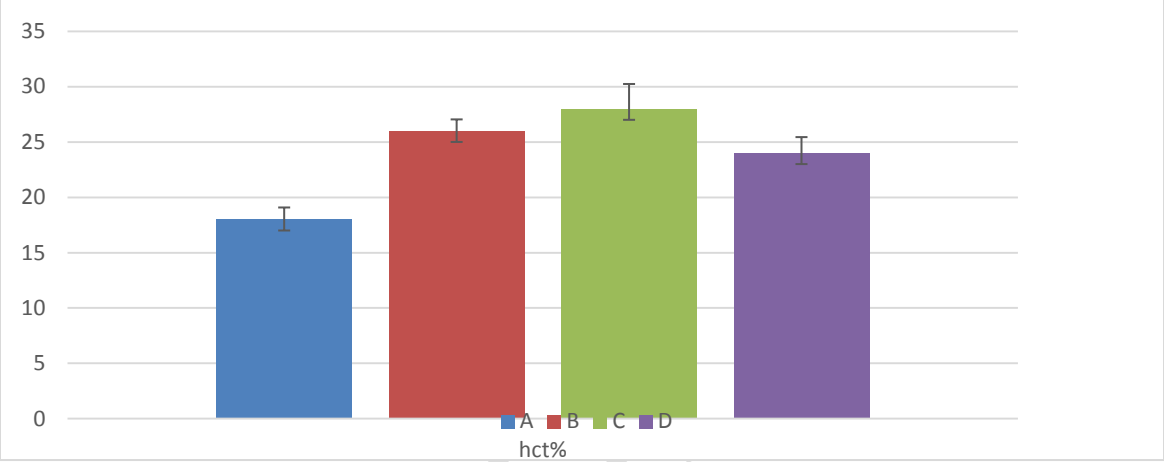


FIGURE 6: PERCENTAGE HAEMATOCRIT OF INDUCED RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group B - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group C - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group D - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

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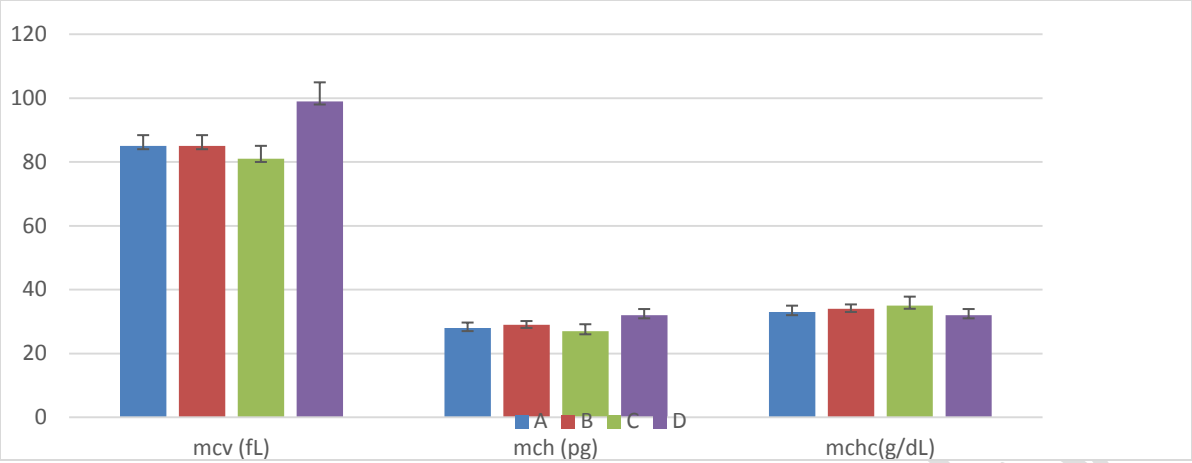


FIGURE 7: RED CELL INDICES OF NORMAL RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A- (Saline substitute)
Group B- 250 mg/kg Polyherbal mixture
Group C- 500 mg/kg Polyherbal mixture
Group D- 750 mg/kg Polyherbal mixture

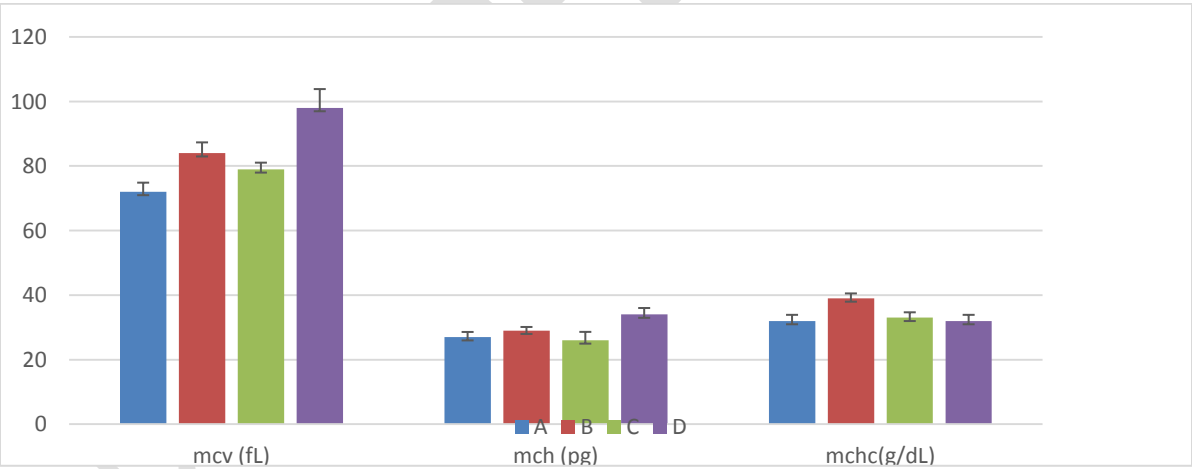


FIGURE 8: RED CELL INDICES OF INDUCED RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group B - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group C - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

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Group D - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

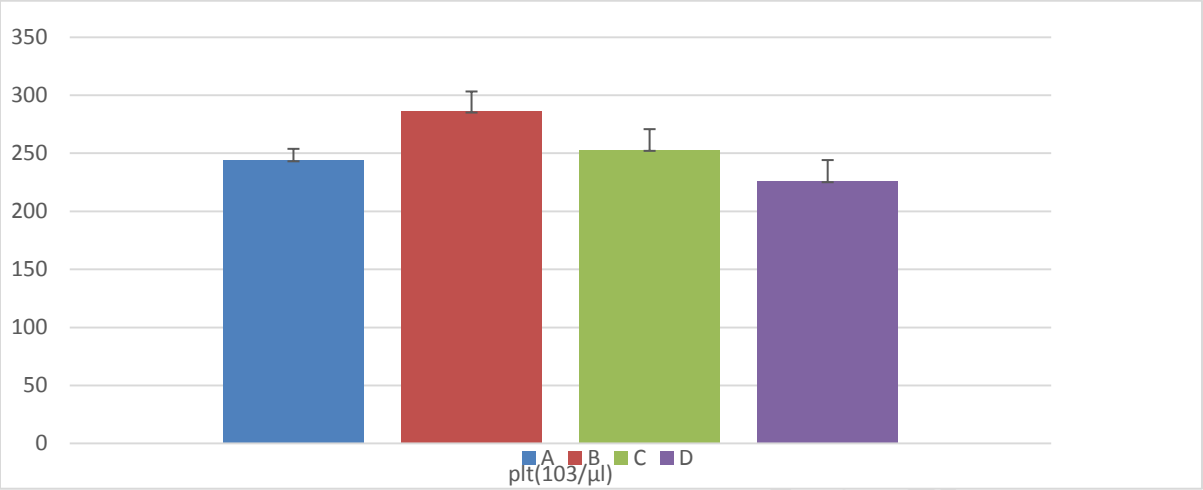


FIGURE 9: PLATELET COUNTS OF NORMAL RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A- (Saline substitute)
Group B- 250 mg/kg Polyherbal mixture
Group C- 500 mg/kg Polyherbal mixture
Group D- 750 mg/kg Polyherbal mixture

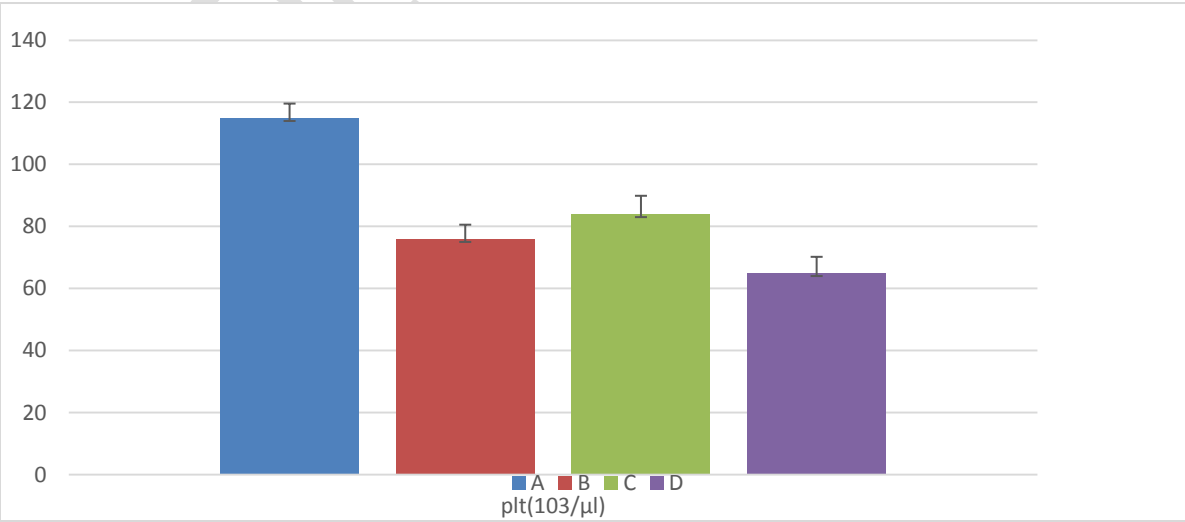

FIGURE 10: PLATELET COUNTS OF INDUCED RABBITS FED/ ADMINISTERED ANTISICKLING POLYHERBAL PREPARATION

Key: Group A - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group B - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group C - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)
Group D - Cadmium (2mg/kg) +10 mg/Kg body weight PHZ (15 days)

## DISCUSSION

Haematological indices remain a sure index in the accessing the health of an individual. Results obtained in this study showed statistically significant increase in WBC count in the normal and induced rabbits and in tandem with the study conducted by Umar *et al*., (20) using *Momordicacharantia* in rats who also reported elevated WBC count when compared to control group. Findings in this research are also in line with a study by Taiwo *et al*., (21) who demonstrated that *P. amarus* contained in this study possess beneficial immunological properties in albino rats. The statistically significant increase in the normal and induced rabbits in the treatment groups compared to control group might be due to the metabolic extracts of the plants which might have resulted in some toxic effects on the body system, and consequently, increased WBC count or increased immunity (20). Decrease in WBC reflects a fall in the production of defensive mechanism to combat infection (22). Animals with high WBC counts are capable of generating antibodies in the process of phagocytosis, hence have high degree of resistance to

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disease while animals with low count are often exposed to high degree of infection (23).

This study also showed statistically significant increase in platelet count of normal rabbits in group B (250mg/kg) and C (500mg/kg) but statistically significant decrease was seen in group D (750mg/kg). Increased platelet activation is another known component of haemostatic activation in patients with SCD. This increased percentage of platelets is activated during steady state in patients with SCD, and this accelerates during vaso-occlusive crisis (VOC) (24). Since increased platelet activation is recognized in patients with sickle cell disease (SCD). This increase in platelets count is an indication that the polyherbal mixture or a component of the mixture sponsors haemopoiesis especially thrombopoiesis. This extract might have stimulated the common myeloid progenitor cells in the bone marrow causing them to differentiate into promegakaryocytes and then into megakaryocytes. However, there is a significant decreased in platelets levels in the induced rabbits. This decrease could be due to increase aggregation of platelets as a result of the high haemoglobin concentration causing increase turbidity and slow flow of blood. A study by Helms *et al*., (25) demonstrated that aggregation of platelet can cause decrease platelet count.

With the exception of RBC of normal rabbits in group D (750 mg/kg polyherbal mixture), RBC, HGB and HCT showed increased levels across all groups of

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normal rabbits administered the polyherbal mixture. This could be an indication that it promotes erythropoiesis. Studies by Ogwumike (26); Taiwo *et al*., (21) have demonstrated that some plant extracts contained in the mixture used for this study have some haematological advantage and favors erythropoiesis. This could be the reason for the increased count in the treatment groups compared to the control group.

This study also shows a similar response in the anaemia induced rabbits but with statistically significant increase in RBC of group B (250mg/kg polyherbal mixture), HGB of group C (500mg/kg) and D (750mg/kg), HCT of group B, C and D. It is seen in the study that treatment in group C (500mg/kg) showed statistically significant increase in RBC, HGB and HCT indicating the concentration resulted in more effect meaning it is dose-dependent (i.e the effect of the polyherbal mixture increases the above parameters in a dose dependent manner). A statistically insignificant decreased level was seen in group D of normal rabbits. This could be correlated to a report by Bawala *et al*., (27) who stated that low haematological values such as RBC, PCV and Hb could be due to harmful effects of high dietary content. A study by Oyenike *et al*., (10) demonstrated that the plants used in this study were able to prevent a good percentage number of sickled cells during induced hypoxia. Hence, the mixture has anti-sickling effect and could

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protect patients with sickle cells from the effects of low oxygen tension especially in state of crisis.

MCV shows statistically significant increase in group C of normal rabbits. MCV and MCHC of induced rabbits showed statistically significant increase in group B and C. increase erythropoietic activities can results in increase MCV thus this increase could be attributed to the established erythropioetic effect resulting in production of reticulocytes and haemoglobinization (28). MCV, MCH and MCHC together with parameters such as red blood cell count and packed cell volume are used in characterizing and diagnosing anaemia as well as establishing blood disorders (29,30,5). The MCV finding in this study showed clinically insignificant increase in group D but showed clinically significant decrease in group C of normal rabbits compared to control group administered saline substitute. Decreased MCV is in line with a study by Ogwumike (26) who reported decreased levels of MCV across the treatment groups of albino rats treated with aqueous extract of the leaf sheath of Sorghum bicolor. However, the findings in the anaemia induced rabbits contrast this finding where MCV shows increased levels. This could be due to the fact that whereas this study was conducted using a polyherbal mixture, the decrease in MCV is induced by extract of *Sorghum bicolar* only. The increase in MCV is a further proof and reflection of a release of macrocytes (large immature erythrocytes) following treatment with the polyherbal mixture (31).

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Hence the statistically significant increase in anaemia induced rabbits could be due to compensatory mechanism.

By this study, the effect of the polyherbal mixture observed shows varying responses appearing to be dose dependent. Each concentration affected the blood counts differently, but mostly in a dose- dependent manner. The changes in the haematological parameters didn't occur simultaneously with increased concentration of the polyherbal mixture but rather increased concentrations gave increased values in some parameters while decreased in some.

## CONCLUSION

The use of polyherbal mixtures in the management of sickle cell disease seem promising. The findings of this study suggest a positive effect (response) on the haemoglobin, red blood cells, packed cell volume and white blood cell count of the rabbits in a dose dependent manner. Increased production of red blood cells, hemoglobin could help combat anaemia and the evidenced leukopoeisis could be of immunological advantage, boosting the immune system, aiding in better immune response. Platelets showed increased response in some groups of the normal rabbits, decreased count was evidenced in the induced rabbits and could be due to the fact that increased platelet activation has been recognized in patients with sickle cell disease (SCD). Hence, if the same effect could be exhibited in

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humans, the polyherbal mixture if compounded in the right dose, could produce some therapeutic effect and aid in the management of SCD.

**Ethical Approval:**

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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