Studies on Erythropoietic Effect of Medicinal Plants on Phenylhydrazine Induced Anemic Rats
9.1 **INTRODUCTION**

The formation of blood cells inside the body is called haemopoiesis, which is a continuous process and erythropoiesis refers to the formation of erythrocytes. The process occurs in myeloid tissue present in the red bone marrow of the humerus, femor, ribs, sternum, pelvis, and portions of the skull. Erythropoiesis is an extremely active process that requires several metabolites for synthesis of haemoglobin, which contains globin as protein part and haem as prosthetic group (Guyton and Hall, 1998). Anemia is a hematological condition with a quantitative deficiency of circulating haemoglobin, accompanied by a reduced number of erythrocytes or red blood cells (RBCs). Erythrocytes play a vital role in body through the supply of oxygen to all the parts of body, the role of RBCs in the body cannot be underestimated (Kumar and Clark, 2001). Anemia, a symptom of many pathological conditions is irritating and uncomfortable and shortens the longevity of the individual if condition persists without treatment. The WHO estimates the number of anemic people worldwide to be staggering 2 billion (about 30% of the world population) and that approximately 50% of all anemias can be attributed to iron deficiency and in resource-poor areas, this is frequently exacerbated by infectious diseases, malaria, worm infestation, and AIDS (WHO, 2011). Traditional medicine is still the mainstay of about 75-80% of world population, mainly in the developing countries. India, having a rich tradition of folk medicine from centuries, has provided very simple but effective remedies to various ailments using plants and plants derived compounds. There is no such risk factor to use the plant medicine as compare with the allopathic drugs. There are several reviews related to evaluation of medicinal plants and their probable role in erythropoiesis.

Ogwumike (2002) reported hemopoietic effect of aqueous extract of the leaf sheath of sorghum bicolor in albino rats. Luay et al (2004) evaluated erythropoietic effects of *Teucrium polium* leaves extract on rats during three weeks of administration and investigated blood picture and some biochemical parameters. They reported the significant decrease in red blood corpuscles (RBCs), hemoglobin (Hb), and packed cell volume (PCV) under the impact of *T. polium* in experimental rats. Lohar et al (2009) investigated the erythropoietic activities of some medicinal plants found in Satpuda region of Jalgaon district in Maharashtra. They evaluated the effect of *Aegel marmelos, Asparagus recemosus, Boerhavia diffusa, Carissa congesta, Eugenia jambolana, Ficus*
carica, Phoenix sylvestris, Phyllanthus emblica, Spinaca oleracea, and Vitis vinifera on Wistar albino rats so far their erythropoietic effect. The result indicated that the RBC count (the highest of 44.96±0.08 for fruit extract Phyllanthus emblica vs. 3.96±0.03 for control) and Hb% (the highest of 13.44±0.18 for fruit extract Phyllanthus emblica vs. 10.74±0.12 for control) in the test animals showed augmentation as compared to the controlled group of rats.

Pawar et al (2010) studied efficacy of Asteracantha longifolia Nees. (Family-Acanthaceae), which is a wild herb commonly used in traditional ayurvedic medicine as Kokilaaksha and the Unani drug as Talimakhana in India and Sri Lanka for various medicinal uses as aphrodisiac, tonic, sedative and blood diseases etc. and reported erythropoietic potential in experimental rats. Qian et al (2011) demonstrated that the Salidroside (which is an active ingredient of Rhodiola rosea) promotes erythropoiesis and protects erythroblasts against oxidative stress by up-regulating glutathione peroxidase and thioredoxin. Rhodiola rosea is commonly used in China and Tibet folk medicine for the treatment of high altitude sickness, anoxia and mountain malhypoxia. This study attempted to examine the potential erythropoiesis-stimulating and anti-oxidative effect of SDS in TF-1 erythroblasts. The erythropoiesis-promoting effect was determined by treating human TF-1 cells, one of the popular in vitro models for studying erythropoiesis, with SDS in the presence and absence of erythropoietin (EPO) through the measurement of the expression of a series of erythroid markers such as glycophorin A (GPA), transferrin receptor (CD71) and hemoglobin (Hb). The potential protective effect of SDS against H2O2-induced apoptosis and its underlying mechanism in TF-1 erythroblasts were examined by flow cytometry and Western blot analysis. SDS promotes erythropoiesis in the EPO-treated cells and it also reduces the number of apoptotic cells in TF-1 erythroblasts after H2O2-treatment probably through the up-regulation of protective proteins thioredoxin-1 (Trx1) and glutathione peroxidase-1 (GPx1). The present study provided evidence to explain the ethnopharmacological role of SDS and Rhodiola rosea in Chinese medicine. The findings also supported the use of SDS as an erythropoiesis-adjuvant agent to correct anemia and malhypoxia.

Aimola et al (2011) tested Terminalia catappa extract for its potential for erythropoiesis in adult Balb C Mice. They reported that the extract showed 16.67% induction of the PCV level of the mice close to Folic acid, which revealed a 25% increase in PCV of the
mice on the other hand the untreated control showed an almost steady PCV level during the 7-day experimental period. This could be important in the management of sickle cell anemia since anaemia as revealed by a much lowered PCV levels in sickle cell anaemia patients is one of the major ways in which the disease is expressed.

Agomuo, et al (2012) carried out studies on the leaves and fruits of Persea Americana to identify its potential for Vitamin, amino acid and haematological parameters. Results obtained for vitamins, showed higher concentrations of niacin, ascorbic acid and tocopherol in the studied fruits than leaves. Aside serine and tyrosine, other amino acids investigated in both samples were appreciably high. Rats placed on leaves and fruits of the studied sample showed insignificant (p>0.05) effect in levels of RBC, Hb, and WBC when compared to those of the control. Neutrophils, lymphocytes, MCH, and MCHC were significantly affected (p<0.05) in test rats against those of the control rats. The study has shown that consumption of leaves or fruit of P.americana may induce a hypochromic or normocytic condition in the body.

Martin and Anna (2012) studied the effect of Ruellia Praetermissa extracts on Erythropoiesis in Pregnant Women. The effect of the extracts of Ruellia praetermissa Schweinf. ex Lindau. on hemoglobin (Hb), Hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV). Mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBC), was investigated in 50 Pregnant women attending prenatal clinic in Belo maternity (Cameroon). The women were assigned to 5 groups of 10 women per group. The first group was the control and the other 4 were the experimental groups. The control was administered daily, 0.5 ml of saline solution while the experimental groups were administered daily oral doses of the plant extract in concentrations of 200 mg/kg, 400 mg/kg, 800 mg/kg and 1,600 mg/kg respectively for 16 days. Blood samples were collected on the 17th day and analyzed. The extracts contain flavonoids aglycones (luteolin and apigenin) and their respective, glycosides and a high concentration of triterpenes (campesterol, stigmasterol, sitosterol, lupeol) and iridoid glycosides. It was also found to be rich in vitamins and minerals. The extracts increased the values in a dose dependent manner Hb (P < 0.05), RBC (P < 0.05), hematocrit (P < 0.05). It however showed no remarkable effect on the values of MCH and MCHC (P > 0.05) but with a dose depending decreasing effect on MCV (P < 0.05). The active principles of this plant drug stimulate erythropoiesis, which leads to increase in
circulating RBCs with slightly microcytic sizes (MCV), normochromic weight, (MCH) normochromic hemoglobin contents (MCHC). The result of this study thus supports the traditional use of *Ruellia praetermissa* in pregnancies threatened with miscarriage and as a remedy for anemia.

Aimola et al (2013) studied erythropoietic and bone marrow stimulating activity of *Terminalia catappa* extract in experimental mice. They reported that *T. catappa* was able to up-regulate the synthesis of intracellular hemoglobin (0.135 ±0.004 µmol/0.1ml) significantly comparable to hydroxyurea (HU) (0.158±0.006 µmol/0.1ml), and normalize the peripheral blood reticulocyte index significantly at *P*<.05 0.94±0.25% close to the non anemic mice 0.97±0.25% and bone marrow carbonic anhydrase activity. TC inhibited arginase activity significantly (*P*<.05) comparable to hydroxyurea. The results demonstrated that *Terminalia catappa* extract as an erythropoietic agent that supports normal erythroid differentiation *in vivo* in phenylhydrazine- induced anemic mice in a synergistic fashion.

As part of continued research and to add knowledge to the existing limited literature on *Cassia fistula*, *Psida cordifolia*, and *Aegel marmelos*, the present study investigated the erythropoietic effect of leaves extract of *A. marmelos*, *C.fistula* and *P.cordifolia* using rat model.

9.2 MATERIALS AND METHOD

Studies on erythropoietic activity of *Cassia fistula*, *Psida cordifolia*, and *Aegel marmelos* was carried out in following ways:

**a) Collection of Plant material and preparation of leaves extract**

The leaves of *Cassia fistula*, *Psida cordifolia*, and *Aegel marmelos* were collected and processed as per discription given in previous chapters. The dried leaves of each plant were ground into a fine powder and a crude extraction with water and 70% (v/v) ethanol was performed. The solution was filtered after 24 h and the filtrate concentrated to a semi-solid form using a rotary evaporator. The material was weighed and solutions of 150 mg/ml were prepared and used for evaluating their erythropoietic potential.
b) Animal used

Adult Wistar rats weighing around 180-200g were purchased from Wockhardt Research Laboratory, Aurangabad, MS, India. The animals were kept in polypropylene cages at an ambient temperature of 25±2°C and 55-65% relative humidity 12±1 hr light and dark schedule was maintained in the animal house till the animals were acclimatized to the laboratory conditions, and were fed with commercially available rat chow (Hindustan Lever Ltd., Bangalore, India) and had free access to water. The experiments were designed and conducted in accordance with the guidelines of institutional animal ethical committee of College of Pharmacy, Chopda Dist. Jalgaon.MS.

c) Experimental design

The studies were conducted in the nine groups with six animals in each group.

Group I: Normal rats provided with normal feed and water (Control).

Group II: Phenylhydrazine induced anemic rats (Experimental)

Group III: Phenylhydrazine induced anemic rats fed with extract of A.marmelos

Group IV: Phenylhydrazine induced anemic rats fed with extract of C.fistula

Group V: Phenylhydrazine induced anemic rats rats fed with extract of P.cardifolia

Group VI: Phenylhydrazine induced anemic rats fed with mixture of extracts from A.marmelos and C.fistula

Group VII: Phenylhydrazine induced anemic rats fed with mixture of extracts from A.marmelos and P.cardifolia

Group VIII: Phenylhydrazine induced anemic rats fed with extract of mixture of extracts from C.fistula and P.cardifolia.

Group IX: Phenylhydrazine induced anemic rats fed with extract of mixture of extracts from A.marmelos, C.fistula and P.cardifolia.

Group X: Phenylhydrazine induced anemic rats fed with Bioferon syrup as reference hematinic. Bioferon syrup (Saha Biologicals, Kolkata) is to treat anaemia in children and adults. 5ml of syrup contains 200mg ferric ammonium citrate (equivalent to 41 mg
elemental iron), 500 µg folic acid, 5 µg vitamin B\text{12} (cyanocobalamin) and 1.75g sorbitol. The dose of 5 ml Bioferon orally administered daily to experimental rats in the study.

Phenylhydrazine (Himedia, Mumbai, 20mg/kg i.p. daily for three consecutive days as described by Berger (1985) to induce anemia in rats Anemic rats were fed with plant extract in dosages of 150 mg/kg body weight to assess therapeutic effect of the extracts. Separate batches of rats were maintained in each group to evaluate effect of individual or combined extracts of plants under study.

d) Blood sample collection

At the end of the feed and water administration periods (7 days), rats from the various groups were weighed and sacrificed after being put to sleep in a closed container with chloroform. Blood collected by direct cardiac puncture into heparin treated tubes. The tubes were properly labelled and used for haematological analysis.

e) Haematological tests

Haemoglobin percentage (Hb%) and were determined using Sahi’s and (Alexander. and Griffith 1993a) methods respectively. Westergreen’s method was used for erythrocyte sedimentation rate (ESR), Counting chamber methods were used for total count of Red blood corpuscles (RBC) and white blood cell total count (WBC Total). Haematocrit method was used for packed cell volume (PCV) whereas, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined as described by Alexander and Griffith (1993b).

The values of RBC, hematocrit (PCV), and haemoglobin test were used to calculate the RBC indices [MCV measured in femtoliter (fl) MCH measured in pictogram (pg) and MCHC measured in gram per deciliter) using following formulae:

\[
\begin{align*}
\text{a)} & \quad \text{MCV (fl)} = \frac{\text{PCV} (\%) \times 10}{\text{RBC} (\text{millions} / \text{mm}^3)} \\
\text{b)} & \quad \text{MCH (pg)} = \frac{\text{Hb} (\text{g}/100\text{ml}) \times 10}{\text{RBC} (\text{millions} / \text{mm}^3)} \\
\text{c)} & \quad \text{MCHC (g/dl)} = \frac{\text{RBC} (\text{millions} / \text{mm}^3) \times 100}{\text{PCV} (\%)}
\end{align*}
\]
f) Statistical analysis:

Values are expressed as mean ± SEM from 6 animals. The statistical significance of difference in the mean and SEM was analysed by student’s t-test for comparison between each of the test groups and the control.

Table 9.1 Erythropoietic effect of aqueous and ethanolic leaves extracts of A. marmelos, C. fistula and P. cordifolia on phenylhydrazine induced anaemic rats of different groups (III to IX) and hematological parameters of normal (I), Anaemic (II) and reference haematinic (X) rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>RBC (x 10^6 / mm^3)</th>
<th>Hb (gm/dl)</th>
<th>ESR (mm/hr)</th>
<th>WBC (x 10^3 / mm^3)</th>
<th>PCV (%)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal)</td>
<td>5.432 ±1.24</td>
<td>12.52 ±1.26</td>
<td>8.29 ±0.98</td>
<td>7.42 ±1.19</td>
<td>39.13 ±1.02</td>
<td>72.03 ±4.24</td>
<td>23.04 ±1.72</td>
<td>31.99 ±2.31</td>
</tr>
<tr>
<td>II (Anaemic)</td>
<td>3.985 ±0.89</td>
<td>7.02 ±1.08</td>
<td>5.04 ±0.08</td>
<td>10.32 ±1.04</td>
<td>36.02 ±0.45</td>
<td>85.37 ±10.3</td>
<td>18.71 ±0.96</td>
<td>19.49 ±1.03</td>
</tr>
<tr>
<td>III AE</td>
<td>4.077 ±0.54</td>
<td>8.68 ±1.22</td>
<td>7.74 ±1.09</td>
<td>7.14 ±0.85</td>
<td>36.21 ±1.02</td>
<td>88.81 ±5.6</td>
<td>21.30 ±1.44</td>
<td>23.97 ±2.04</td>
</tr>
<tr>
<td>EE</td>
<td>4.332 ±1.13</td>
<td>8.82 ±1.05</td>
<td>8.65 ±0.76</td>
<td>8.07 ±1.25</td>
<td>36.89 ±1.34</td>
<td>85.16 ±6.4</td>
<td>20.36 ±1.05</td>
<td>23.90 ±1.46</td>
</tr>
<tr>
<td>IV AE</td>
<td>3.783 ±1.32</td>
<td>7.98 ±0.98</td>
<td>6.98 ±0.79</td>
<td>8.06 ±1.18</td>
<td>34.56 ±0.94</td>
<td>91.35 ±4.5</td>
<td>21.09 ±0.78</td>
<td>23.09 ±0.95</td>
</tr>
<tr>
<td>EE</td>
<td>3.941 ±0.82</td>
<td>8.65 ±1.43</td>
<td>7.74 ±1.28</td>
<td>8.89 ±1.07</td>
<td>36.43 ±1.32</td>
<td>92.43 ±6.8</td>
<td>21.94 ±1.45</td>
<td>23.74 ±2.32</td>
</tr>
<tr>
<td>V AE</td>
<td>4.784 ±0.94</td>
<td>9.99 ±1.53</td>
<td>7.88 ±1.25</td>
<td>7.73 ±0.82</td>
<td>37.88 ±0.94</td>
<td>79.18 ±5.3</td>
<td>20.88 ±0.92</td>
<td>26.37 ±1.44</td>
</tr>
<tr>
<td>EE</td>
<td>5.224 ±0.78</td>
<td>12.87 ±1.62</td>
<td>9.17 ±0.89</td>
<td>8.98 ±1.42</td>
<td>39.01 ±1.09</td>
<td>74.67 ±4.7</td>
<td>24.63 ±2.33</td>
<td>32.99 ±2.43</td>
</tr>
<tr>
<td>VI AE</td>
<td>4.465 ±0.66</td>
<td>8.58 ±1.25</td>
<td>8.54 ±1.04</td>
<td>7.66 ±0.76</td>
<td>35.75 ±1.32</td>
<td>80.08 ±5.6</td>
<td>19.21 ±1.21</td>
<td>24.0 ±2.04</td>
</tr>
<tr>
<td>EE</td>
<td>4.788 ±1.24</td>
<td>9.07 ±0.95</td>
<td>8.92 ±0.67</td>
<td>7.82 ±0.98</td>
<td>36.08 ±1.45</td>
<td>75.35 ±4.9</td>
<td>18.94 ±1.06</td>
<td>25.14 ±3.55</td>
</tr>
<tr>
<td>VII AE</td>
<td>5.032 ±0.93</td>
<td>10.35 ±1.57</td>
<td>8.73 ±1.09</td>
<td>8.62 ±0.74</td>
<td>35.36 ±0.89</td>
<td>70.27 ±6.5</td>
<td>20.57 ±1.45</td>
<td>27.70 ±2.34</td>
</tr>
<tr>
<td>EE</td>
<td>5.311 ±0.68</td>
<td>12.76 ±1.85</td>
<td>9.34 ±0.89</td>
<td>9.87 ±1.05</td>
<td>38.85 ±1.05</td>
<td>73.16 ±5.7</td>
<td>23.49 ±2.12</td>
<td>32.84 ±3.21</td>
</tr>
<tr>
<td>VIII AE</td>
<td>4.201 ±0.55</td>
<td>8.35 ±1.08</td>
<td>7.78 ±1.32</td>
<td>7.95 ±0.67</td>
<td>36.12 ±1.47</td>
<td>85.98 ±7.4</td>
<td>19.88 ±0.76</td>
<td>23.18 ±1.34</td>
</tr>
<tr>
<td>EE</td>
<td>4.487 ±1.13</td>
<td>9.35 ±1.21</td>
<td>8.07 ±0.79</td>
<td>8.65 ±1.07</td>
<td>37.21 ±0.91</td>
<td>79.39 ±6.3</td>
<td>20.83 ±1.54</td>
<td>25.12 ±1.66</td>
</tr>
<tr>
<td>IX AE</td>
<td>4.77 ±0.76</td>
<td>9.54 ±1.52</td>
<td>6.89 ±1.08</td>
<td>8.04 ±0.59</td>
<td>36.88 ±0.63</td>
<td>77.31 ±6.2</td>
<td>20.0 ±0.96</td>
<td>25.87 ±1.96</td>
</tr>
<tr>
<td>EE</td>
<td>4.93 ±1.34</td>
<td>10.22 ±1.56</td>
<td>8.35 ±1.4</td>
<td>9.21 ±1.06</td>
<td>37.68 ±0.76</td>
<td>76.43 ±5.8</td>
<td>20.73 ±2.05</td>
<td>27.12 ±2.05</td>
</tr>
<tr>
<td>X (Haematinic)</td>
<td>5.11 ±0.55</td>
<td>11.97 ±0.39</td>
<td>7.71 ±1.04</td>
<td>9.83 ±0.95</td>
<td>38.54 ±1.11</td>
<td>75.42 ±3.4</td>
<td>23.42 ±2.11</td>
<td>31.09 ±1.25</td>
</tr>
</tbody>
</table>

Each value is Mean±SD of six observations. AE= Aqueous extract. EE=Ethanolic extract.
9.3 RESULT

The observed data given table 9.1 shows that the ethanolic leaves extracts of *Cassia fistula*, *Psida cordifolia*, and *Aegel marmelos* were more potent in erythropoietic activities in phenylhydrazine induced anaemic rats than that of aqueous extracts of plants selected for study. The hematological parameters including total count of RBC and WBC, Hb%, ESR, PCV, MCV, MCH and MCHC of phenylhydrazine induced anaemic rats (group II) were significantly differ from the values of these parameters in normal rats (group I). After administration of phenylhydrazine to experimental rats (group II), there were very significant reduction (P<0.001) in total count of RBC (percent reduction: 28% over its normal value), Hb (-44%), ESR (-40%), PCV (-18%), MCH (-19%) and MCHC (-39%) at the same time there was considerable increase in total number of WBC (+39%) and MCV (18%).

After 7 days of treatment, 150 mg/kg ethanolic leaf extracts of *A. marmelos*, *C.fistula*, and *P.cordifolia* showed significant increment (P<0.01 to P<0.001) in RBC count, Hb%, and ESR in of phenylhydrazine induced anaemic rats proving their erythropoietic potency. In group III anaemic rats treated with ethanolic leaves extract of *A.marmelos*, RBC count increased from 3.985 ±0.89 x10^6 /mm^3 to 4.332 ±1.13 x10^6 /mm^3 and Hb% increased from 7.02 ±1.08 to 8.82 ±1.05 gm/dl. This significant increment was at P<0.01. Somewhat similar erythropoietic effect was noted in rats of group IV anaemic rats provided ethanolic leaves extract of *C.fistula* as well as in group VI anaemic rats treated with mixture of ethanolic leaves extracts of *A.marmelos* and *C.fistula*. Interestingly, anaemic rats (of group V) treated with ethanolic extract of *P.cardifolia* showed highly significant (P<0.001) erythropoietic effect since their RBC count increased upto 5.311 ±0.68 x10^6 /mm^3 from 3.985 ±0.89 x10^6 /mm^3 and Hb% increased from 7.02 ±1.08 to 12.87 ±1.62 gm/dl.

On comparing the result of effect of mixture of ethanolic leaves extracts of plants selected for the study indicated that group VII anaemic rats treated with mixture of ethanolic leaves extracts of *A.marmelos* and *P.cardifolia* showed highly significant (P<0.001) erythropoietic effect since their RBC count increased upto 5.311 ±0.68 x10^6 /mm^3 from 3.985 ±0.89 x10^6 /mm^3 and Hb% % increased from 7.02 ±1.08 to 12.76 ±1.85 gm/dl. Group VII and IX anaemic rats also showed significant (P<0.01) erythropoietic potency but it less than the effect showed by the group VII anaemic rats.
The standard hemitinic drug Bioferon also showed significant (P<0.001) erythropoietic effect in group X rats. In present part of investigation, there was significant increase in total count of WBC in anaemic rats of group (II) and rats of group III to X tend to maintain its level at higher values than that of normal rats (I). There were no significant changes in the values of PCV, MCV MCH, and MCHC in experimental rats than that of normal rats.

9.4 DISCUSSION

Phenylhydrazine induced anaemic rats showed significant reduction in total count of RBCs due to its hemolytic action on erythrocytes as a consequence there was reduction in Hb%. Phenylhydrazine induced hemolysis of RBCs may generates toxic free radicals that can attack macromolecules like haemoglobin resulting in oxidative damage within red blood cells and oxidative degradation of spectrin in the cell membrane skeleton leading to its distuction. In addition, free radical attack accelerates the normal aging process of RBCs causing premature splenic sequestration (Berger, 1985 and 2007). This resulted in quantitative reduction of circulating RBCs, Hb% and ESR.

Comparatively, highly significant increase in RBC count and Hb% with slightly microcytic sizes (MCV), normochromatic weight, (MCH) normochromic hemoglobin contents (MCHC) in anaemic rats treated with ethanolic extracts of *P cardifolia* on individual basis and in combination with *P cardifolia* and *A marmelos* imply that *P cardifolia* could possibly have some components vitamins B\textsubscript{12} (cyanocobalamin) and minerals like iron and copper which play indispensible role in erythropoiesis. Cyanocobalamin is the major maturation factor necessary for erythropoisis, iron is necessary for the formation of the heme part of the haemoglobin and copper is important for the absorption of iron from the gastrointestinal tract (Sembulingam and Prema, 2010) Whereas in combined form there may be synergetic action of erythropoietic components present in *P cardifolia* and *A marmelos* that lead to boost the process of formation of erythrocytes in endothelial tissue including bone marrow. Resembling results in hematological profile were recorded by Koffuor, et al (2012) during their studies on erythropoietic effect of root bark extract of *Carissa edulis* in phenylhydrazine induced anemic rats.
On the other hand, white blood cells exhibit a significant increase in the count (P <0.001). This can be attributed to the reactive response due to the stress that affects the bone marrow and is demonstrated by the inhibition of erythropoiesis and thrombopoiesis (Dessypris, 1999). The elevated WBCs seen with the induction of phenylhydrazine could also be due to body’s defence mechanism of getting rid of hymolytic products of RBCs destruction (Lohar et al, 2009; Aimola et al, 2013).

9.5 CONCLUSION

The present study has revealed that phytochemicals present in *P.cardifolia* and *A.marmelos* could have contributed to faster and dose-dependant reversal of anaemia in experimental rats during treatment compared to normal rats. The effect of body’s homeostatic mechanism at reversing the induced anaemia could be delayed by the effect of phenylhydrazine that could still linger or awhile. The plant components of *P.cardifolia* and *A.marmelos* could by acting in synergy to elicit their haematopoetic and erythropoietic effects but this would also require further isolation, purification and characterization. These findings provide the pharmacological basis for the traditional use of these plants for therapeutic use in the management of anaemia. The safety for use however needs to be ascertained in toxicity studies.