British Journal of Pharmaceutical Research
4(21): 2563-2574, 2014
ISSN: 2231-2919

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Characterization of Bioactive Components of *Gossypium barbadense L.* with Hematinic Potential in Wister Albino Rats

Zara Muhammad[1], Samuel Atabo[2*] and Amina Zakari[1]

[1]Department of Applied Science, College of Science and Technology, Kaduna Polytechnic, Kaduna, Nigeria.
[2]Department of Biochemistry, University of Agriculture, Makurdi, Benue State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author ZM designed the study, performed the statistical analysis and wrote the protocol. Author SA managed the experimental analyses of the study and wrote the first draft of the manuscript. Author AZ managed animals and carried out the literature searches.

Article Information

DOI: 10.9734/BJPR/2014/13194
Editor(s):
(1) Syed A. A. Rizvi, Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, USA.
Reviewers:
(1) Anonymous, Federal University of Santa Maria (UFSM), Brazil.
(2) Anonymous, Sfax University, Tunisia.
(3) Anonymous, Monash University Malaysia, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=788&id=14&aid=6870

Original Research Article

Received 7th August 2014
Accepted 1st October 2014
Published 6th November 2014

ABSTRACT

Aims: To ascertain the hematinic potential and bioactive compounds in *Gossypium barbadense.*
Place and Duration of Study: Department of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, and Department of Applied Science, College of Science and Technology, Kaduna Polytechnic, Kaduna, Nigeria between February, 2013 and July, 2014.

**Corresponding author: Email: atabosam@yahoo.com;*

Methodology: Forty eight (48) apparently healthy albino rats weighing (150-200g) were grouped in to seven groups of five rats each. Thirteen rats were used for the *G. barbadense* toxicity test. Hemolytic anemia was induced using Phenylhydrazine (10mg/kg bw). Different doses (100mg/ml, 200mg/ml, and 400mg/ml) of *G. barbadense* were administered with periodic evaluation of Haematological indices (Hemoglobin concentration, Packed Cell Volume, Red Blood Cells and reticulocyte count). Bioferon (0.23ml/kg b.w) was used as the standard drug. Synergistic Thin layer Chromatography and Column Chromatography were used to purify the plant extract. Gas Chromatography linked with Mass Spectroscopy (GC-MS) was used in the Characterization of purified fraction.
Results: The level of Hb (g/dl) was found to increase in a dose dependent manner (100mg-12.17g/dl, 200mg-12.60g/dl and 400mg-12.87 g/dl), likewise RBC (4.31, 4.41 and 4.72) and PCV (43.35%, 43.49%, 43.65%). Characterization revealed the presence of 19 compounds.
Conclusion: *G. barbadense* resulted in HB, RBC and PCV boost, owing to inherent bioactive component.

Keywords: Gossypium barbadense; haemolytic anaemia; Phenylhydrazine.

1. INTRODUCTION

Anaemia is one of the clinical conditions that constitute a serious health problem in many sub-Saharan countries as a result of the prevalence of different forms of parasitic infections, caused by *plasmodium, trypanosome* and *helminthes* [1]. Prolonged use of non-steroidal anti-inflammatory drugs as well as exposure to toxic chemicals such as phenyl hydrazine has also been implicated to cause the condition [2-4]. Anaemic condition is characterized by a decrease in the level of circulating hemoglobin, less than 13 g/dl in male and 12 g/dl in [5]. In the tropics, due to endemicity of malaria and other parasitic infections, between 10 to 20% of the population were reported to possess less than 10 g/dl of Hb in the blood [6]. Anaemia can reduce the work capacity of an individual or entire population, bringing serious economic consequences and obstacles to national development [7]. Hematinic substances are essential for the proper formation of the component of blood.

The cotton plant is an annual herb belonging to the genus *Gossypium* of the *Malvaceae* family. There are four *Gossypium* species; *G. herbaceum L.*, *Gossypium arboreum L., G. hirsutum L.* and *G. barbadense L.* [8]. *Gossypium barbadense L.* typically has a longer growing period, and produces smaller bolls that give a significantly low yield [8]. *Gossypium barbadense L.* has been reported to have lots of therapeutic effects [9-11] mostly attributed to its active constituent “gossypol” [12]. The traditional use of *G. barbadense* among indigenes of moduganari in Borno State, Nigeria is prominent. It is prepared by decoction. Depending on the age of the patient, about five centiliters of the preparation is consumed morning and night for duration of five to seven days. This study is intended to scientifically investigate the trado-medical assertion of its potency as a haematinicand to elucidate its specific bioactive components.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Samplecollection

The *Gossypium barbadence* leaves were collected from Moduganari, area of Maiduguri, (Borno state, Nigeria) and authenticated at the Herbarium section of the Department of Biological Science, Ahmadu Bello University, Zaria, with a voucher no. 453.

2.1.2 Equipment / chemicals/ reagents

Column chromatography apparatus, GC-MS analyser (GC-MS-QP2010 PLUS SIMAZU, JAPAN). Phenylhydrazine was purchased from sigma chemical company, Paderborn - Germany. Other chemicals used where of analytical grade.

2.1.3 Animals

Forty-Eight (48) apparently healthy male and female albino rats (150-200g) were used for this study. They were housed in well-ventilated cages, controlled temperatures (28℃ - 31℃); humidity (40 – 65%), and a daily light cycle of 13 to 15 hours was strictly observed. Other factors that were taken very seriously included sanitation, bedding and vermin control. Animals were fed with commercial rat feed –grower's mash (containing protein, carbohydrate, mineral, fiber, vitamins and fat) and allowed to acclimatize for two weeks before the commencement of the study. The weekly feed intake and body weight of the animals has been recorded until the end of the study.

2.1.4 Preparation of methanolic extract

Leaves of the cotton were shade dried and ground to powder using mortar and pestle. Dried powdered plant material (500 g) was extracted with 2 L of methanol by cold extraction method for 24 hours in large amber bottles with intermittent shaking. At the end of the extraction, the crude methanol extract was filtered and the filtrate concentrated by evaporation (at 45℃).

2.2 Methods

2.2.1 Determination of LD_{50}

The LD_{50} was carried out by the method described by [13].

In the first phase nine rats were divided into 3 groups of 3 rats each and then treated with the methanolic extract of the plant at doses of 10 mg, 100 mg and 1000 mg/kg body weight (bw) intraperitoneally. They were observed for 24hr for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with plant extracts at doses of 1000 mg, 1600 mg, 2900 mg, and 5000 mg/kg body weight intraperitoneally. The median lethal dose (LD_{50}) was calculated as the square root of the products of the highest non-lethal dose and the lowest lethal dose.

2.2.2 Experimental design

Haemolytic anaemia was induced in groups II to VI (GII-GVI) by a daily intraperitoneal injection of 10 mg/kg Phenylhydrazine, for four successive days, according to Dhakar, (2012). GI and GVII constituted the control groups and were similarly treated using the commercial diet and the plant extract respectively. The experimental rats were monitored for a period of twenty-one (21) days, after the final injection.

Group I: Normal control, fed with only commercial diet and water,
Group II: Negative control, given distilled water (1 ml) with neither drug nor plant extract,
Group III: Positive control, treated with Bioferon (0.23ml/kg b.w)
Group IV: Given 100mg of methanolic extract per Kg bw,
Group V: Given 200mg of methanolic extract per Kg bw**,**
Group VI: Given 400mg of methanolic extract per Kg bw,
Group VII: Given only commercial diet, water and extract (Extract control).

2.2.3 Haematological investigation

The haematological indices red blood cell (RBC) count was estimated by visual counting improved by neubauer counting chamber. Hb and PCV were determined using cyanomethamoglobin and microhematocrit methods respectively [14].

2.2.3.1 Packed cell volume (PCV)

Packed cell volume was determined by microhematocrit method as described by [14] using microhematocrit centrifuge. PCV was carried out by obtaining blood from a slightly cut rat tail and allowing the blood to flow through a capillary tube to more than two-third of the tube. The end with blood will then be sealed off using plasticine. The capillary tubes will be placed in the centrifuge and centrifuged for 15 min at 3,000 rpm. The capillary tubes will then be placed on the microhemotocrit to determine the packed cell volume.

2.2.3.2 Hemoglobin concentration

Haemoglobin concentration (Hb) will be carried out using the cyanomethaemoglobin method of [14].

2.2.3.3 Red blood cell and white blood cell count

These will be estimated using improved Neubauer counting chamber [14].

The mean cell volume (MCV), mean cell haemoglobin (MCH) and the mean cell haemoglobin concentration (MCHC) were calculated employing the formula of [15].

Mean Cell Volume: The mean cell volume (MCV) will be determined as

$$MCV = 10 X \frac{\text{Hamatocrit}}{\text{Red Blood Cell Count}}$$

Mean Cell Hemoglobin Mean Corpuscular Hemoglobin (MCH) will be calculated as follows

$$MCH = 10 \times \frac{\text{Haemoglobin}}{\text{Red Blood Cell Count}}$$

Mean Corpuscular Hemoglobin Concentration (MCHC): It will be calculated as follows

$$MCHC = \frac{\text{Haemoglobin}}{\text{Hamatocrit}} \times 100$$

2.2.4 Thin layer chromatography (TLC)

Commercially prepared TLC aluminum sheets of 20 x 20cm lined with silica gel was used. The plate was cut to size of 5 x 5cm and the extract spotted at the bottom of the TLC plate (about 0.5cm from the base). The plate was placed in a developing tank containing chosen solvent system. The initial solvent system used is 100% hexane. The polarity of solvent system was increased by adding ethanol at various ratios of 95:5, 90:10, 85;15, 80:20 until 50:50 ratio of hexane:ethanol respectively was achieved. The spots were developed using iodine vapor and viewed under UV light.

2.2.5 Partial purification of crude extract

Partial purification was carried out using a modified method of [16]. In this method, the crude extract of *G. barbadense* was purified using silica gel packed in a chromatographic column. Slurry of finely powdered silica gel (in hexane) was packed in glass column to a height of about 12cm and loaded with 10mg/ml of the extract dissolved in ethanol. It was separated by gradient elution using solvent that shows better resolution from an initial TLC of extract. Different fractions were collected at intervals of 1hour, although, where a different colour band is observed, it was collected in to a different beaker, until there appears to be no more solute in the column. The fractions collected were monitored by TLC and similar fractions (i.e fraction with the same retention time) were pulled together. Solvent in each flask was allowed to evaporate at room temperature. TLC analysis was carried out on the semi dry bulk fraction.

2.2.6 Characterization of purified fraction

Components of the purified extract were characterized (further separation, analysis and identification) using Gas Chromatography linked Mass Spectroscopy (GC-MS).

2.3 Statistical Analysis

The results were expressed as Mean ± Standard Deviation (SD). The data were analyzed using the analysis of variance (ANOVA) and the differences between means were compared using the Duncan multiple range test.

3. RESULTS

3.1 Lethal Dose of Methanolic Extract of *G. barbadense* Leaves ($LD_{50\ (IP)}$)

The ip LD_{50} was calculated to be 2154.07mg/kg bw. About 5%, 10% and 20% (of LD_{50}) dose of the extract was used for treatment as low, medium and high (doses) respectively.

3.2 Effect of Methanolic Extract of *G. barbadense* on Some Hematological Parameters

Table 1, showed the effect of Intraperitoneal administration of 10mg/kg b.w Phenylhydrazine (PHZ), for 4 days on Hb concentration, PCV, RBC Counts, Reticulocyte count, MCV, MCH and MCHC. The Hb concentration in all PHZ – induced animals were significantly ($p<0.05$) lowered after 4 days. Except for groups I and VII that were not anaemia-induced, groups II ,III, IV, V and VI showed Hb concentration of 11.43±0.46, 11.40±0.75, 11.40±0.75, 11.56±0.58 and 11.38±0.42 respectively, which are indicative of anaemia. Also significant reduction in RBC and rapid increase in the amount of reticulocytes concomitantly represents the induction of anaemia.

The group treated with 100 mg/kg b.w (GIV) showed an increase in Hb concentration after 7 days, 14 days and 21 days (11.85±0.66, 11.99±0.64, and 12.17±1.30, $P<0.05$). The groups treated with 200 mg/kg bw (Grp V) had a Hc of 12.60±0.76 g/dl after three weeks treatment with *G. barbadense.* The group that received 400mg/kg b.w of the extract showed the highest level of recovery and a relative improvement compared to the untreated animals ($P<0.05$) at the third week of the experiment, as shown in Tables 2, 3 and 4.

Though the level of Hb recovery was observed to be progressive (from week 1 to week 3) across all the treated animals, the highest was at the 3rd week. It was also observed that the recovery was distinctly dose-dependent as the 400mg dosage showed the highest effect on Hb concentration at the end of the experiment. The RBCs at the fourth day showed to be 4.06±0.14 in GVI which turns out to be 4.72±0.22 at the third week after treatment. The PCV also rose to 43.65±0.16 at week 3 from 42.97±0.18 after day four. Conversely, reticulocyte count diminished (10.78±0.46 at day four to 9.86±1.40 at 21 days), $P<0.05$, with increasing extract dose during the 21 day period (Tables 2, 3 and 4). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) represent traditional morphological parameters.

3.3 Characterization of Purified Methanolic Extract of *Gossypium barbadense*

Characterization of purified extract was carried out using Gas Chromatography linked Mass Spectroscopy (GC-MS). Table 5 revealed the major components of *Gossypium barbadense* extract, their molecular weight, chemical formula and retention index.

4. DISCUSSION

When rats were injected intraperitoneally with PHZ there was decrease in RBC, Hb concentration and PCV, but the reticulocyte count was increased. The increase in reticulocyte count was a functional response to systemic need for mature RBCs, thus indicative of animal-model-induced haemolytic anaemia.

This agrees with the findings of [17] who induced anaemia in rats following a single PHZ intraperitoneal administration at a dose of 20 mg/kg b.w. (aqueous solution): erythrocyte count lowered to about 50% and haemoglobin level to about 60% of normal values for days after PHZ injection. Similar results were obtained in this study when experimental rats were administered PHZ in order to induce anaemia. In addition, [18] observed increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats.

Table 1. Haematological indices showing the Effect of Intraperitoneal Administration with PHZ (10mg/kg B.W) daily for 4 days

	Grp I	Grp II	Grp III	Grp IV	Grp V	Grp VI	Grp VII
Hb (g/dl)	19.05±0.25	11.43±0.46	11.40±0.75	11.45±0.20[a]	11.56±0.58[a]	11.38±0.42[a]	19.18±0.15
RBC (10^6/µL)	7.22±0.17	4.09±0.12	4.28±0.09	3.94±0.09[a]	4.00±0.12[a]	4.06±0.14[a]	7.33±0.38
PCV (%)	49.08±0.60	49.08±0.14	42.95±0.48	42.72±0.59[a]	42.89±0.14[a]	42.97±0.18[a]	48.99±0.27
Ret. (%)	1.99±0.67	10.87±0.87	10.94±0.69	11.06±0.64[b]	11.20±0.78[b]	10.78±0.46[b]	2.36±0.88
MCV	67.97±2.19	105.00±2.91	99.93±3.22	109.02±3.19[a]	107.45±3.50[a]	105.81±3.40[a]	66.95±3.37
MCH	26.37±0.43	27.97±1.88	26.67±2.13	29.09±0.94[a]	28.90±1.89[a]	28.02±1.46[a]	26.22±1.44
MCHC	38.82±0.83	26.62±1.10	26.69±1.84	26.69±0.76[a]	26.89±1.34[a]	26.48±1.01[a]	39.16±0.49

Each value represents Mean±SD. Superscripts 'a' = P<0.05, 'b' = 0.01, when compared with Normal Control (Grp I)
Key: Grp I – Normal control, Grp II - Negative control (Distilled H_2O), Grp III – Positive control (Bioferon 0.23ml/kg b.w), Grp IV – low dose (100mg), Grp V – medium dose (200mg), Grp VI – high dose (400mg), Grp VII – extract control (Grp 7)

Table 2. Hematological indices of PHZ induced hemolytic anaemic rats treated with methanolic extract of *G. barbadense* leaves and bioferon for one (1) week

	Grp I	Grp II	Grp III	Grp IV	Grp V	Grp VI	Grp VII
Hb (g/dl)	19.15±0.23	11.16±0.41	12.05±0.48	11.85±0.66[a*]	12.14±0.34[a*]	12.00±0.79[a*]	19.94±0.57
RBC (10^6/µL)	7.24±0.19	3.91±0.12	4.44±0.14	3.96±0.12[a]	4.01±0.07[a]	4.25±0.11	7.44±0.40
PCV (%)	49.19±0.73	42.78±0.20	43.10±0.47	42.97±0.31[a]	43.05±0.27[a]	43.18±0.21	49.10±0.27
Ret. (%)	2.02±0.34	11.57±0.58	10.76±0.47	10.73±1.07[b*]	10.98±0.75[b*]	10.78±1.51[b*]	2.36±0.88
MCV	68.00±2.32	109.49±3.77	97.11±2.94	108.53±3.8[b*]	107.49±2.13[b*]	101.64±2.51[b*]	66.14±3.43
MCH	26.47±0.86	28.57±1.45	27.17±1.77	29.96±2.26[a]	30.31±0.96[a]	28.26±2.18[a]	26.90±2.26
MCHC	38.93±0.13	26.09±0.97	27.96±1.31	27.58±1.39[a]	28.20±0.86[a]	27.79±1.86[a]	40.62±1.29

Each value represents Mean±SD. Superscripts 'a' = P<0.05, 'b'=P<0.01 when compared with Normal Control (Grp I), '' =P< 0.05, when compared with Day 4, Key: Grp I – Normal control, Grp II - Negative control (Distilled H_2O), Grp III – Positive control (Bioferon 0.23ml/kg b.w), Grp IV – low dose (100mg), Grp V – medium dose (200mg), Grp VI – high dose (400mg), Grp VII – extract control (Grp 7)*

Table 3. Hematological indices of phz induced hemolytic anaemic rats treated with methanolic extract of *G. barbadense* leaves and bioferon for two (2) weeks

	Grp I	Grp II	Grp III	Grp IV	Grp V	Grp VI	Grp VII
Hb (g/dl)	19.36±0.28	10.89±0.34	12.56±0.45	11.99±0.64^{a*}	12.49±0.77^{a*}	12.67±0.69^{a*}	20.31±0.75
RBC (10^6/µL)	7.32±0.20	4.26±0.13	4.68±0.17	4.09±0.10^{a}	4.18±0.13^{a}	4.41±0.16^{a*}	7.62±0.38
PCV (%)	49.28±0.73	42.81±0.21	43.33±0.31	43.04±0.31^{a}	43.12±0.16^{a}	43.38±0.21^{a*}	49.19±0.22
Ret. (%)	2.13±0.44	11.87±0.97	10.23±0.69	10.55±1.04^{a}	10.64±0.84^{a}	10.43±1.49^{a}	2.33±0.35
MCV	67.29±1.89	100.61±2.95	92.70±3.97	105.36±3.40^{a*}	103.28±3.15^{a*}	98.46±3.09^{a*}	64.66±3.21
MCH	26.43±0.65	25.62±1.48	26.87±1.65	29.36±2.10^{a}	29.90±1.85^{a}	28.75±1.92^{a*}	26.74±2.30
MCHC	39.28±0.35	25.45±0.81	28.98±1.02	27.85±1.37^{a}	28.97±1.85^{a}	29.20±1.59^{a}	41.30±1.58

Each value represents Mean±SD. Superscripts 'a' = P<0.05, 'b'=P<0.01 when compared with Normal Control (Grp I), '' =P< 0.05, when compared with Day 4, Key: Grp I – Normal control, Grp II - Negative control (Distilled H_2O), Grp III – Positive control (Bioferon 0.23ml/kg b.w), Grp IV – low dose (100mg), Grp V – medium dose (200mg), Grp VI – high dose (400mg), Grp VII – extract control (Grp 7)*

Table 4.Hematological indices of PHZ induced hemolytic anaemic rats treated with methanolic extract of *G. barbadense* leaves and bioferon for three (3) weeks

	Grp I	Grp II	Grp III	Grp IV	Grp V	Grp VI	Grp VII
Hb (g/dl)	19.54±0.19	10.82±0.36	13.25±1.13	12.17±1.30^{a*}	12.60±0.76^{a*}	12.87±0.59^{a*}	20.63±0.71
RBC(10^6/µL)	7.42±0.19	4.30±0.15	4.99±0.17	4.31±0.10^{a*}	4.41±0.07^{a*}	4.72±0.22^{a*}	7.75±0.38
PCV (%)	49.38±0.74	42.87±0.21	43.61±0.82	43.35±0.47^{a*}	43.49±0.25^{a*}	43.65±0.16^{a*}	49.45±0.18
Ret. (%)	2.05±0.13	10.87±0.77	10.07±0.53	10.18±0.99^{a*}	10.25±0.71^{a*}	9.86±1.40^{a*}	1.74±0.50
MCV	66.60±1.76	99.79±3.33	87.51±3.70	100.74±3.27^{a*}	98.55±1.95^{a*}	92.68±4.24^{a*}	63.96±3.18
MCH	26.35±0.65	25.21±1.60	26.61±2.63	28.32±3.41^{a*}	28.55±1.81^{a*}	27.33±1.72^{a*}	26.72±2.20
MCHC	39.57±0.34	25.25±0.87	30.40±2.68	28.07±2.83^{a*}	28.97±1.85^{a*}	29.49±1.35^{a*}	41.73±1.50

Each value represents Mean±SD. Superscripts 'a' = P<0.05, 'b'=P<0.01 when compared with Normal Control (Grp I), '' =P< 0.05, when compared with Day 4, Key: Grp I – Normal control, Grp II - Negative control (Distilled H_2O), Grp III – Positive control (Bioferon 0.23ml/kg b.w), Grp IV – low dose (100mg), Grp V – medium dose (200mg), Grp VI – high dose (400mg), Grp VII – extract control (Grp 7)*

Table 5. Results of GC-MS

S/No	Name of compounds	Mol. Wt.	Chemical formula	Retention index (RI)
1	1,1 Dimethybutanol	102	$C_6H_{14}O$	709
2	(3 - Ethyl-2 oxirany) ethanone	114	$C_6H_{10}O_2$	806
3	(3 - Ethyl - 2 oxirany) ethanone	114	$C_6H_{10}O_2$	806
4	4,11,11 – Trimethyl – 8 – methylene Bicycle (7.2.0) undec - 4 - ene	204	$C_{15}H_{24}$	1494
5	9 - octadecene	252	$C_{18}H_{36}$	1818
6	Hexadecene	226	$C_{16}H_{34}$	1612
7	1- (1,5 - dimethyl - 4 - hexany) 4 - methyl - 3 -Cyclohexen-1-ol	222	$C_{15}H_{26}O$	1619
8	9-Eicosene	280	$C_{20}H_{40}$	2017
9	Hexadecanoic acid, methyl ester palmitic acid	270	$C_{17}H_{34}O_2$	1878
10	3-Eicosene	280	$C_{20}H_{40}$	2017
11	9,12,15-Octadecatrienoic acid, methyl ester linolenic acid	292	$C_{19}H_{32}O_2$	2101
12	Phytol-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	2045
13	Oleic acid 9-octadecanoic acid	282	$C_{18}H_{34}O_2$	2175
14	1-Tricosene	322	$C_{23}H_{36}$	2298
15	Hexadecanoic acid	691	$C_{37}H_{74}NO_8P$	0
16	Pentaflouropropionic acid heptadecyl ester	402	$C_{20}H_{35}F_5O_2$	1872
17	13-octadecenal	266	$C_{18}H_{34}0_4$	2007
18	Di-n-octylphthalate 1,2-Benzene dicarboxylic acid	390	$C_{24}H_{38}O_4$	2832
19	Squalene 2,6,10,15,19,23-hexamethyl	410	$C_{30}H_{50}$	2914

PHZ causes haemolysis by interacting with sulfhydryl groups, the inhibition of various enzymes, immune mechanisms, and the fragmentation of erythrocytes as they pass through the platelet-fibrin mesh by unknown or yet to be defined mechanisms [19].

The results of this present study demonstrated that the administration of methanolic extract of *G.barbadense* induced significant increase in RBC, Hb, PCV levels and a decrease of the reticulocyte count of PHZ induced anaemia.

A relative and gradual dose dependent recovery of the haemolytic condition induced by phenylhydrazine was observed.

A significant correlation with diagnostic values has been demonstrated between RBC, Hb, PCV and the RBC indices (MCV, MCH and MCHC) in both humans and rats [20-21]. In this study administration of PHZ to rats also resulted in a significant increase in the MCV and MCH values, which are indicators of macrocytosis thus describing the anaemia as macrocytic. This condition is also common in Vit B_{12} and folate deficiencies probably as a result of iron deficiency [22]. Macrocytic anaemia has also been reported in rats infected with *Trypanosoma brucei brucei* [23] and this has been linked to iron deficiency anaemia [24]. The presence of macrocytosis reduced towards normal as the rats recovered from the anaemic condition. There was no macrocytosis in situation of megaloblastic, aplastic, dyserythropoietic and sideroblastic anemias [20,23]. MCH and MCHC values between normal and PHZ–induced rats are not being significantly different indicating that PHZ-

induced anemia is not a hypochromic or spherocytic anemia. This agrees with the findings of [21,25].

Earlier studies [26] revealed the presence of mineral elements (Fe, Mg, K, Na, Zn) and phytochemicals in *G. barbadense,* which was linked with improvement in hematological indices. We therefore propose that the presence of these anti-oxidant mineral element and phytochemicals may account for the protection of both the haematopoietic committed stem cells and the blood cells from the attack of the reactive free radicals generated by PHZ, thus promoting the synthesis of RBC, as a means of alleviating anemia. This is in concordance with the findings of Samuel [27] who suggested that a synergistic interplay between phytochemicals and mineral element plays a significant role in improving anemia.

5. CONCLUSION

The current study has shown that the extract of *Gossypium barbadense* L. has haematinic potential, and its trado-medical use in the treatment of anaemia is not out of place. The research also provides prospective bioactive compounds to look out for, in haematinic and anti-anaemic studies.

CONSENT

Not applicable.

ETHICAL APPROVAL

This work was carried out on the standard principles for animal care. All authors hereby declare that the laboratory animals were treated most humanely as examined and approved by appropriate supervisors.

COMPETING INTEREST

The authors declare that there is absolutely no conflict of interest regarding the publication of this article.

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