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ORIGINAL ARTICLE

Haematopoietic effect of an ethanolic leaf extract of *Ipomoea involucrata* P. Beauv in phlebotomized New Zealand White Rabbits

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Ipomoea involucrata **is used by some religious bodies (that do not believe in the use of allopathic medicine) in Ghana to treat haematological conditions particularly anaemia. The aim of this study therefore is to determine the haematopoietic effect of the ethanolic leaf extract of *Ipomoea involucrata* to establish the scientific bases for its use. The haematological profile of healthy New Zealand rabbits, phlebotomized rabbits, and phlebotomized rabbits treated with 0.23 ml/kg Feroglobin®, 100, 300, and 1000 mg/kg *I. involucrata* ethanolic leaf extract, or 0.23 ml normal saline were determined at 20-day intervals for 40 days using the Cell Dyn 1800 Automatic Analyzer®. Data obtained was analyzed using GraphPad Prism version 5. The 300 and 1000 mg/kg dose of the extract and the reference hematinic caused significant increments ($P \leq 0.01$ -0.001) in white blood cells, red blood cells, haemoglobin concentration, hematocrit, and platelets counts within 20 days of treatment. The mean corpuscular volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration, and the red cell distribution width recorded in all categories were not significantly different. This indicates that the ethanolic leaf extract of *Ipomoea involucrat*a has some haematopoietic activity and thus could be effective in managing anaemia and other blood cell deficiency disorders.**

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INTRODUCTION

Anaemia, the most predominant blood cell deficiency disorder, is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development (de Benoist *et al.,* 2008). It occurs at all stages of life, but is more prevalent in pregnant women and young children. More frequently it coexists with a number of other causes, such as malaria, parasitic infection, nutritional deficiencies, and haemoglobinopathies (de Benoist *et al.,* 2008). Other blood cell deficiency disorders are neutropenia and thrombocytopenia.

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Severe anaemia (prevalence exceeds 2%) is a problem in most countries in Africa and South Asia and some countries in East Asia and the Pacific (Galloway, 2003; Stoltzfus, 2003). In most parts of Africa the main causes of anaemia are usually due to poor nutrition and malaria. An earlier study has shown prevalence of anaemia in southern Ghana to be fairly common, particularly in children and women (Commey and Dekyem, 1995).

Low income levels in Ghana (Obeng, 2008) pose a challenge in the affordability of orthodox haematinics. Access to health centers in rural areas is also a problem; meanwhile as much as 80 % of people in developing countries are said to depend on traditional medicines for primary healthcare (Bodekar, 1994; WHO, 2008). In fact, the Alma Ata declara-

tion of 1978 encouraged the use of all available resources for primary healthcare and recommended that government should give high priority to using traditional health practices and incorporate proven traditional remedies into National Drug Policy and Regulations (Zhang, 1994). One widely used traditional remedy for anaemia is *Ipomoea involucrata*.

Ipomoea involucrata P. Beauv.*var. involucrata* (Family: Convolvulaceae) commonly known as Dutchman's pipe is a slender but vigorous, sprawling or twining annual or perennial herb, of grassland, secondary scrub and forest origin very common throughout tropical Africa. Its several medicinal uses are as follows: an infusion of the whole plant is drunk as a stimulant, or preventative of fever; a decoction of the fresh sap is taken as a remedy for gonorrhoea; the leaves are used for asthma (Oliver, 1960); a plant preparation is added to baths or made into a lotion for treating jaundice (Bouquet & Debray, 1974); the leaf-sap is applied and rubbed into areas of localized edema and is instilled into the eyes for filarial infection; an aqueous decoction is taken by women for dysmenorrhea and at child-birth to hasten expulsion of the after-birth; and a compress of pounded up stems is used for headache (Bouquet, 1969).

It is in this light that this study has been conducted to determine the haematopoietic effect of *Ipomoea involucrata* to establish the scientific bases for its use and to add to the armamentarium of herbs that can be used to manage anaemia in Ghana.

MATERIALS AND METHODS

Plant Collection

Ipomoea involucrata was collected from KNUST campus in August 2008 and authenticated by Mr. G. H. Sam of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana where a voucher specimen with number KNUST/HM1/2011/R004 has been deposited. The leaves were harvested, air dried, and powdered using a hammer mill (Schutte Buffalo, New York, USA).

Preparation of Extract

A 220 g quantity of the leaf powder of *Ipomoea involucrata* was soxlet extracted with 70 % ethanol. The liquid extract obtained was condensed under low temperature and pressure using a Buchi Rotor Evaporator (Rotavapor R-210, Switzerland), and further dried over a hot water bath (B-Tex Laboratory Engineering, Gujarat India) at 40 ^{o}C. The dried extract weighing 18.5 g (percentage yield: 8.4) was labeled and stored in a desiccator. Required quantities taken and dissolved in distilled water for use in this study will be referred to as the ethanolic extract of *Ipomoea involucrata* or EIE.

Phytochemical Screening

The ethanolic leaf extract of *Ipomoea involucrata* was subjected to phytochemical screening in accordance with the standard procedure (Harborne 1998).

Animals and Husbandry

Male New Zealand White Rabbits (4.8 – 5.2 kg) obtained from Kwadaso Agricultural College, Kumasi, Ghana were kept in the Department of Pharmacology, KNUST, animal house for this study. They were fed with normal rabbit chow (GHAFCO, Tema, Ghana), fresh herbs like *Aspilia Africana* and rough meadow-grass (*Poa trivialis*) from around the KNUST campus, and clean water. During the experimental period, the rabbits were kept in clean, well-ventilated, individual, front-opening stainless steel cages (that had grid plastic floor) in the laboratory under ambient dark-light cycle and relative humidity. The rabbits were allowed to acclimatize to the laboratory environment for one week prior to experimentation. During this period, the animals were periodically assessed for gross morphological and behavioral changes.

All procedures and techniques used in these studies were in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals (NIH, Department of Health Services publication No. 83-23, revised 1985). The protocols for the study were approved by the Departmental Ethics Committee.

Reference Haematinic

Feroglobin® (Vitabiotics Ltd, Great Britain), a liquid tonic containing Iron 0.2 %, Zinc 0.06 %, Cop-

per 0.02 %, Manganese 0.025 %, and vitamin B-Complex 0.39 % in a blend of honey and malt, was used as the reference hematinic. The dose of Feroglobin® administered to experimental animals in this study is equivalent to the adult human dose (0.23 ml/kg/day) stated by the manufacturers.

Phlebotomization of Rabbits

Phlebotomization of the New Zealand White rabbits was achieved by following a rabbit bleeding protocol (McGuill and Rowan, 1989) after their basal haematological profile had been determined using the Abbot Cell Dyn 1800 Automatic Analyzer® (Abbott Diagnostics, USA). In this protocol, the rabbits were sedated using sodium thiopentone (60 mg/kg, i.p). The left ears were shaved to expose the auricular artery (which runs medially in the ear). This was disinfected with 70% ethanol and rubbed gently with 30% limonene in 95% ethanol to dilate it. A hypodermic needle (22, or 20 gauge, 1 inch long) attached to a calibrated tube was inserted into the central artery about two-thirds the way up the central artery, with the tip of the needle pointing towards the base of the ear (McGuill and Rowan, 1989). About 15-20 % of the total volume of blood, calculated based on 60 ml/kg per rabbit (Suckow *et al.,* 2002), in the rabbit were collected into the calibrated tube. When blood collection was completed, the needle was removed and the artery held off firmly with cotton wool for 3 minutes until the bleeding stopped. Drops and smears of blood were cleaned with 70% ethanol to prevent infection of punctured site. The rabbits were returned to the cage when there was no further bleeding which is indicative of restored circulation. Phlebotomization was considered satisfactory and normocytic anaemia was considered induced when blood cell counts reduced by at least 30% after the haematological profile were determined after 24 hours.

Experimental Procedure

The phlebotomized rabbits were weighed and put into five groups (A-E) with five animals per group for the study. The groups were treated with 0.23 ml/kg Feroglobin®, 100, 300, or 1000 mg/kg EIE, or 0.23 ml/kg normal saline for 40 days. A sixth group, F, with 5 healthy rabbits was also kept under the laboratory conditions but these were not given any form of treatment. At day 20 and day 40, blood samples from animals in all groups were collected into MediPlus K3 EDTA tubes (Sunphoria Co. Ltd., Taiwan) for haematological analysis using the Abbot Cell Dyn 1800 Automatic Analyzer®. Data obtained on white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), and platelet count (PLT) were recorded for analysis.

Statistical Analysis

Significant changes in measured parameters between normal, phlebotomized, and phlebotomized but treated rabbits were determined using One-Way Analysis of Variance followed by Dunnet's Multiple Comparison Test. Statistical estimates were made at a confidence limit of 95% and probability values ($P \leq 0.05$ were considered significant. The analysis was done using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA).

RESULTS

Phytochemical Screening

Results from the phytochemical screening of EIE is as shown in Table 1.

Table 1: Results obtained from phytochemical screening of ethanolic extract of *Ipomoea involucrate* (EIE)

Components	EIE
Tannins	+
Flavonoids	-
Alkaloids	+
Sterols	-
Glycosides	+
Saponins	+
Triterpenoids	-

(+) indicates the presence of; (-) indicates the absence of the phytochemicals tested

Phlebotomization

There was a very significant reduction ($P \leq 0.001$) in RBCs, HGB, HCT and PLT after phlebotomy. The WBC was also significantly ($P \leq 0.01$) reduced; however, there were insignificant changes ($P > 0.05$) in MCV, MCH, MCHC, and RDW (Table 2).

Haematopoietic effect

Treatment of phlebotomized rabbits with 300 mg/kg EIE for 20 days resulted in significant increments ($P \leq 0.05$) in WBC, RBCs, and very significant increments ($P \leq 0.001$) in HGB, HCT and PLT. The 1000 mg/kg EIE also caused significant increments ($P \leq 0.05$) in WBC and very significant increments ($P \leq 0.01$) in RBC and PLT. HGB, and HCT were also very significantly ($P \leq 0.001$) elevated. The reference hematinic showed very significant elevation ($P \leq 0.01$) in WBC, RBC, and PLT as well as very significant elevation ($P \leq 0.001$) in HGB and HCT. Changes in MCV, MCH, MCHC, and RDW with treatments were however insignificant (Table 3). After 40 days of treatment, the reference drug-treated and the 1000 mg/kg-treated groups showed very significant increments ($P \leq 0.01$) in WBC, and even more significant increments ($P \leq 0.001$) in RBCs, HGB, HCT and PLT. The 100 mg/kg EIE treatment also showed some significant increments ($P \leq 0.05$) in WBC and PLT and also significant increments in RBC, HGB, and HCT (not seen with the normal saline-treated group). With the exception of WBC which increased significantly ($P \leq 0.05$), RBC, HGB, HCT, and PLT increased very significantly ($P \leq 0.001$) in the 300 mg/kg-treated group. However there were still no significant changes in MCV, MCH, MCHC, and RDW with the treatments (Table 4).

Table 2: The haematological profiles of New Zealand White rabbits prior to phlebotomization (Normal I), phlebotomized rabbits (PBTM), and normal rabbits kept under experimental conditions over the 40-day study period (Normal II).

Variables	Normal I	PBTM	Normal II
WBC (K/μL)	8.7±1.5	5.3±1.7††	8.0±1.8^{ns}
RBC (M/μL)	6.6±0.8	4.0±0.8†††	6.3±0.9^{ns}
HGB (g/dL)	13.2±0.7	8.3±0.8†††	12.9±1.0^{ns}
HCT (%)	42.3±3.5	27.3±3.9†††	40.8±3.6^{ns}
MCV (fL)	64.2±2.3	63.4±3.5^{ns}	64.9±3.2^{ns}
MCH (pg)	21.5±2.6	21.9±3.0^{ns}	22.5±3.6^{ns}
MCHC (g/dL)	33.9±2.3	35.2±2.9^{ns}	34.5±3.5^{ns}
RDW (%)	16.2±1.9	16.6±2.1^{ns}	16.4±1.8^{ns}
PLT (K/μL)	379±55.8	232±43.6†††	342±48.8^{ns}

Values quoted are means ± SD. Levels of significance between phlebotomized/normal II and normal I was determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant decrements: †† implies P ≤ 0.01; ††† implies P≤ 0.001.

DISCUSSION

The significant reduction in the blood cells in circulation after phlebotomy is an indication that this protocol causes blood cell deficiency which will require haematopoiesis for correction. The very significant elevation of WBC, RBC, and PLT caused by the 300 and 1000 mg/kg doses of EIE and the reference hematinic indicates enhanced haematopoiesis. This indicates that EIE can be used to correct anaemia resulting from active bleeding, malnutrition, pregnancy, and chronic disease; leucopenia caused by viral infection, rheumatoid arthritis and autoimmune disorders; and thrombocytopenia caused by diminished platelet survival. Haematopoiesis requires iron and the B-complex vitamins (Koury and Ponka, 2004), folic acid, some mineral such as copper, zinc and manganese, and the action of haematopoietic growth factors or haematopoietic cytokines. Similarity in effect between the higher doses of the extract and the reference hematinic suggests that the extract could have some of the components stated or could possibly stimulate the synthesis and release of the haematopoietic growth factors (which are synthesized by activated cells under required conditions rather than being produced constitutively all the time) to cause the proliferation and differentiation of haematopoietic stem cells of the bone marrow into the various blood cells.

Although the body's homeostatic mechanisms could induce haematopoiesis (seen as the slight elevation of blood cells in the normal saline treated rabbits), the extracts and the reference hematinic induced haematopoiesis very significantly in a

Table 3: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits and phlebotomized rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Feroglobin®, and 100, 300, and 1000 mg/kg EIE for 20 days.

Variables	PBTM	NS	Feroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/µl)	5.3±1.7	6.3±1.4^{ns}	9.0±1.3††	7.2±1.2^{ns}	8.0±1.3†	8.0±1.5†
RBC(M/µl)	4.0±0.8	4.4±0.6^{ns}	5.8±0.6††	4.8±0.7^{ns}	5.4±0.7†	5.6±0.9††
HGB (g/dl)	8.3±0.8	9.0±0.8^{ns}	11.6±0.8†††	9.8±0.7†	10.9±0.7†††	11.3±0.9†††
HCT (%)	27.3±3.9	30.7±2.9^{ns}	39.6±1.9†††	32.1±2.4†	37.0±2.4†††	37.1±1.8†††
MCV (fl)	65.4±3.5	66.8±2.9^{ns}	65.5±3.2^{ns}	65.2±3.5^{ns}	66.8±2.6^{ns}	65.4±3.8^{ns}
MCH (pg)	20.0±1.0	19.5±1.4^{ns}	19.9±1.9^{ns}	19.3±1.1^{ns}	19.8±1.2^{ns}	19.9±1.0^{ns}
MCHC (g/dl)	31.2±2.9	31.6±2.1^{ns}	31.2±2.6^{ns}	31.3±2.4^{ns}	31.7±2.9^{ns}	31.4±2.7^{ns}
RDW (%)	16.6±2.1	16.4±1.7^{ns}	16.4±2.2^{ns}	16.8±1.5^{ns}	16.0±1.9^{ns}	16.6±2.1^{ns}
PLT (K/µl)	232±43.6	278±51.8^{ns}	365±62.7††	291±47.8^{ns}	380±56.2††	388±72.5††

Values quoted are means ± SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies P ≤ 0.05; †† implies P ≤ 0.01; ††† implies P ≤ 0.001; ns implies P > 0.05.

Table 4: A comparison of the haematological profiles of phlebotomized (PBTM) rabbits treated with 0.23 ml/kg normal saline (NS), 0.23 ml/kg Feroglobin®, 100, 300, and 1000 mg/kg EIE for 40 days.

Variables	PBTM	NS	Feroglobin®	EIE (100)	EIE (300)	EIE (1000)
WBC(K/µl)	5.3±1.7	6.9±1.3^{ns}	8.8±1.3††	7.8±1.5†	8.1±1.4†	8.5±1.2††
RBC(M/µl)	4.0±0.8	5.5±0.9†	7.3±0.9†††	5.9±0.9††	6.5±0.7†††	6.8±0.9†††
HGB (g/dl)	8.3±0.8	10.8±1.3†	14.5±1.2†††	11.9±1.7††	13.3±1.1†††	14.1±1.9†††
HCT (%)	27.3±3.9	36.1±3.4††	48.7±3.9†††	40.4±2.8†††	43.7±2.7†††	45.9±3.7†††
MCV (fl)	65.4±2.0	66.2±1.6^{ns}	66.4±1.5^{ns}	66.1±1.7^{ns}	66.7±1.4^{ns}	65.8±1.7^{ns}
MCH (pg)	20.0±1.0	19.3±1.6^{ns}	19.5±1.5^{ns}	19.9±1.2^{ns}	20.1±1.8^{ns}	19.2±1.1^{ns}
MCHC(g/dl)	31.2±2.9	29.8±1.1^{ns}	30.4±1.3^{ns}	30.7±1.4^{ns}	29.9±1.1^{ns}	30.1±1.2^{ns}
RDW (%)	16.6±2.1	16.9±1.9^{ns}	16.1±1.4^{ns}	16.6±2.1^{ns}	16.5±1.7^{ns}	16.2±1.8^{ns}
PLT (K/µl)	232±43.6	303±49.7^{ns}	419±52.2†††	324±48.4†	362±58.6††	388±64.5†††

Values quoted are means ± SD. Levels of significant between the phlebotomized and the phlebotomized but treated rabbits were determined using One-Way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Comparisons Test. For significant increments: † implies P ≤ 0.05; †† implies P ≤ 0.01; ††† implies P ≤ 0.001.

shorter duration of time. The insignificant changes in haematological profile between the normal animals prior to phlebotomy and normal rabbits kept under experimental conditions indicates that the experimental conditions had no adverse effect on the haematological profile of experimental animals.

Preliminary phytochemical screening carried out in this study indicated that *Ipomoea involucrata* leaves contain tannins, alkaloids, glycosides, and saponins in its ethanolic extract. The haematopoietic potential of the leaf extract may be related to its phyto chemicals present. Glycosides and saponins have been documented to significantly increase the proliferation abilities of bone marrow cells (Kirby and Bentley, 1991; Gao *et al.,* 1992; Li *et al.,* 2011). Alkaloids enhance the restoration of haematopoiesis (Boyko and Belskiy, 1998). Tannins have been reported to inhibit the formation of superoxide ions and hydroxy radicals, which are two strong peroxidation agents (Facino *et al*., 1990; Uboh *et al.,* 2010). This antioxidant activity may protect both the haematopoietic committed stem and the formed blood cells from the attack of the reactive free radicals in the body.

CONCLUSION

The ethanolic leaf extract of *Ipomoea involucrata* has some haematopoietic effects and thus can be used to treat anaemia and other blood cell deficiency disorders. Its safety for use however needs to be ascertained.

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