

In Vivo Antianaemic Effect and Safety of Aqueous Extracts of *Erythrina abyssinica* and *Zanthoxylum usambarensis* in Mice Models

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Abstract

This study was carried out to determine the hematinic effects and long term safety of *Zanthoxylum usambarensis* and *Erythrina abyssinica* in mice. Aqueous stem extracts of *Z. usambarensis* and *E. abyssinica* were screened for their haematinic activity in Phenylhydrazine induced anemic mice using the oral route. Hematological parameters were analysed as indices of anemia. The safety of these plant extracts was studied by orally administering 1 g/kg body weight of aqueous extracts daily in mice for thirty days and determining changes in body and organ weight, hematological and biochemical parameters. The mineral content of the extracts was estimated using Total Reflection X-Ray Fluorescence system (TRXF) while phytochemicals were assessed using standard procedures. Phenylhydrazine (PHZ) treatment induced macrocytic anemia. Administration of *Z. usambarensis* and *E. abyssinica* extracts at 100 mg/kg body weight daily for three weeks increased the red blood cell count, hemoglobin, and packed cell volume and decreased the mean cell volume and mean cell hemoglobin though *E. abyssinica* returned these parameters to normal. Administration of *Z. usambarensis* and *E. abyssinica* extracts at 1 g/kg body weight daily for four weeks significantly increased the activities of alkaline phosphatase, gamma glutamyltransferase, lactate dehydrogenase and amylase and the levels of urea and creatine in the treated mice. Extracts from both plants had alkaloids and flavonoids and minerals potassium, calcium, chromium, iron, copper, zinc, manganese, nickel, arsenic, and lead. This study has confirmed *in vivo* haematinic activity and safety of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica*. The observed haematinic activity could be attributed to the phytonutrients present in these plants. The study recommends continued use of *Z. usambarensis* and *E. abyssinica* in the management of anaemia at the right doses as high doses are toxic.

Keywords: Anaemia; *Erythrina abyssinica*; *Zanthoxylum usambarensis*; Alkaline phosphatase; Lactate dehydrogenase

Introduction

Anemia is a blood disorder that is defined as either red blood cell (RBC) count below normal, or red blood cells which are smaller in size than normal or a level of hemoglobin below normal [1]. The various forms of anemia include iron deficiency anemia; hemolytic anemia (destruction of RBCs); vitamin B₁₂ deficiency anemia; folic acid deficiency anemia; anemias caused by inherited abnormalities of RBCs such as sickle cell anemia and thalassemia; and anemia caused by chronic (ongoing) disease [1]. Anemia has attained epidemic proportions worldwide and it impairs normal development in children and constitutes a major public health problem in young children in the developing countries with wide social and economic implications [2,3]. It is a very expensive disorder to manage by use of conventional drugs. Iron deficiency anemia is managed by prescribing iron supplements and or a diet of foods rich in iron. Iron pills are taken together with vitamin C to promote iron absorption. Iron pills are not taken together with antacids and dairy products since these prevent iron absorption by the body. Iron tablets may have side effects such as abdominal cramping; nausea; constipation; and dark, hard stools. Taking the iron

at mealtimes prevents stomach and intestinal upset [4]. Vitamin B₁₂ deficiency anemia is managed by an injection of vitamin B₁₂ once a month or orally taking a high dose vitamin B₁₂ tablet. Folic acid deficiency anemia is managed by daily oral folate tablets. Anemia caused by inherited abnormalities of RBCs is managed using several approaches including intravenous fluids, rest, pain relief, and sometimes blood transfusion. Blood transfusion is associated with the risk of acquiring blood-borne diseases such as hepatitis or AIDS, even though donated blood is screened. People who have thalassemia do not take iron medication. Anemia caused by chronic disease such as chronic kidney disease can be managed by regular injections of erythropoietin to stimulate the body's production of red blood cells [2,3]. In addition to different synthetic drugs, plant remedies and dietary traditions play an effective role in diminishing the suffering due to anemia. The potential role of medicinal plants as hematinic agents is supported by the ethnobotanical surveys and traditional medicines of different cultures [5]. In Kenya, the use of plant extracts has no boundaries for their curative activities and uses, even in the treatment of anemia. Two indigenous plants used in the management of anemia and are known by herbalists in Kenya are *Zanthoxylum usambarensis* and *Erythrina abyssinica* [6]. *Z. usambarensis* (Engl.) Kokwaro is a branched scrub of up to 8 meters high often multi stemmed with spreading crown and dropping branches [7]. It is

commonly referred as Muvuu, Muguchwa, Sagawaita, Roko, Mugucua, Mulasi, Sagawaita, Ol-Oisugi or Loisugi in Kenya. Its bark is greyish brown, branches are deeply fissured with straight or slightly up curved dark red prickles. Leaves have translucent gland dots and toothed margin. It has a hot taste. Its flowers are small and creamy white in colour. It's found in Tanzania, Rwanda, Ethiopia, and Kenya. It is used in house building, for furniture and bow making and also as a medicinal plant [8,9]. In the traditional medicine the bark and roots are used as a cough remedy, serves as an emetic, and are used against malaria while a decoction of the bark is drunk to treat rheumatism [10]. The phytochemicals isolated from *Z. usambarensis* include (+)-tembetarine, (+)-magnoflorine, (-)-edulinine, (+)-N-methylplatydesmine, (-)-blongine, (-)-usambarine, usambanoline, (-)-cis-N-methylcanadine, nitidine, chelerythrine, o-methylcedrellopsin, canthin-6-one, oxychelerythrine, norchelerythrine, pellitronine, (+)-sesamin, and (+)-piperitol-3, 3-dimethylallyl ether [10-12]. *E. abyssinica* Lam. ex DC is a deciduous tree with a short trunk, thick spreading branches and a rounded crown [13]. Its bark is deeply grooved, brown, thick and corky with or without spines. In Kenya it is referred to Mgalla, Omutembe, Muvuti, Murembe, Kakaru, Mbamba ngoma and Muhuti. Leaves are compound with 3 leaflets of which the largest leaflet is rounded to 15 cm. The branches and underside of leaves covered with grey brown hairs. Its flowers are red-orange often appearing when the tree has shed its leaves. The flowers have narrow calyx lobes and the petals are colored [13]. It has woody pods 4-16 cm long which are hairy and strongly narrowed between seeds. The pods contain 1-10 shiny red seeds with a grey black patch. It is found all over Africa in warm temperate and tropical areas as well as Central America, Australia, southern Asia and Hawaii. In Kenya, it occurs in the open woodland or grassland. It is used ornamentally in nitrogen fixation. In Luo community, it is associated with evil spirits and hence not planted in homesteads [8]. It is planted through large cuttings that have been stripped of leaves.

Medicinally, pounded parts are used in a steam form to treat diseases such as anthrax, and the bark is used to treat snakebites, malaria, sexually transmittable diseases such as syphilis and gonorrhoea, amoebiasis, cough, liver inflammation, stomach-ache, colic and measles. The liquid from crushed bark of green stems is used to cure conjunctivitis (inflammation of the eye lids) caused by *Chlamydia trachomatis* (trachoma), whereas bark sap is also drunk as an anthelmintic. The bark is also applied against vomiting. Roasted and powdered bark is applied to burns, ulcers and swellings. A decoction is taken orally as an anthelmintic and to relieve abdominal pains. The roots are used to treat syphilis, and diabetes. Seeds of *E. abyssinica* contain a curare-like poison that if injected into the bloodstream acts as an anaesthetic that causes paralysis and even death by respiratory failure [9,14]. Pounded flowers serve to treat dysentery. A maceration of the flower is drunk as an abortifacient, and applied externally to treat earache. Roots are taken to treat peptic ulcers, epilepsy, malaria, blennorrhagia and schistosomiasis. Leaves are taken to treat peptic ulcers; they are also used for the treatment of diarrhoea. A leaf decoction serves as an emetic. Leaves are applied externally to wounds and painful joints; they are also applied to treat skin diseases in cattle. Fruit extracts are taken to treat asthma and meningitis [8]. The phytochemicals isolated from *E. abyssinica* include 5-prenylbutein, 5-deoxyabyssinin II, licoagrochalcone A, homobutein, octacosylferulate, 3-hydroxy-9-methoxy-10-prenylpterocarpene, 7,4'-dihydroxy-2',5'-dimethoxyisoflav-3-ene, 5-Deoxyabyssinin II, Abyssinin III, abyssinone IV, Abyssinone V, Abyssinone V-4'-methyl ether, Sigmoidin A, Sigmoidin B, Sigmoidin B-4'-methyl ether, Sigmoidin C, Sigmoidin

E, 3-Hydroxy-9-methoxy-10-prenylpterocarpene, 8-methoxyneorautenol, 3-hydroxy-9-methoxy-10-(3,3-dimethylallyl) pterocarpene, 7,4'-Dihydroxy-2',5'-dimethoxyisoflav-3-ene, eryvarin L, erycristagallin and shinpterocarpin [15-18].

Conventional drugs used in the management of anemia are either unaffordable or unavailable and may still have undesirable side effects. While herbal medicines that are in use are cheap and are thought to be readily available and less toxic from most medicinal plants, the use of some plant extracts without evaluation of toxicity could lead to complications or death. Though the medicinal use of *E. abyssinica* and *Z. usambarensis* in the management of anemia is documented, their efficacy and long term safety is unknown [19]. The aim of this study was therefore to determine the hematinic effects and long term safety of these plants in mice.

Materials and methods

Collection of medicinal plants

Six kilograms of each of the plant samples was collected in December 2008 from their natural habitat in Makueni district, Eastern province, Kenya based on ethnopharmacological use. Their ethnopharmacological use was revealed through interviews with local communities and Traditional Health Practitioners (THP). The identity of each of the plants was authenticated by a taxonomist in the Department of Plant and Microbial Sciences, Kenyatta University. A voucher specimen of each of the plants was deposited at the University's Herbarium for future reference. Information gathered included vernacular names (in parentheses) and the part used in preparation of the herbal anti-anaemic remedies.

Drying and processing of plant

The stems were harvested and their barks peeled off while still fresh and cut into small portions and dried at room temperature for one month. The dry stem barks were then ground into powder form using an electric mill (Christy and Norris Ltd., England). The powdered plant material was kept in the dark in a closed plastic container at room temperature.

Preparation of aqueous plant extracts

Five hundred grams of the powdered plant material was extracted at 60°C in four litres of distilled deionized water for three hours. At the end of the extraction time, the extract was decanted into a clean dry conical flask and then filtered through folded cotton gauze and Whitman filter paper grade 1 into another dry clean conical flask. The filtrate was then freeze-dried in 200 ml portions using a Modulyo Freeze Dryer (Edwards, England) for 48 hours. The freeze dried powder was then weighed and stored in an airtight container at -20°C until used for bioassay.

Animals

Three to four week old male white albino mice bred in the Animal House of the Department of Biochemistry and Biotechnology, Kenyatta University were used in this study. These mice weighed on average 25 g. The Animal House in which the mice were bred was maintained at a temperature of 25°C and had 12 hours/12 hours photoperiod. The mice were fed on rodent pellets and water *ad libitum*.

Experimental design

The animal groups involved in this study included the normal mice (the reference group), anemic control mice (the negative control group), anemic experimental mice orally administered with 50, 100 and 350 mg/kg body weight of plant extracts.

Induction of anemic condition

Anemic condition was induced experimentally by an intraperitoneal administration of 5 mg/kg body weight phenyl hydrazine daily from Sigma (Steinheim, Switzerland) to each mouse for seven days. On the eighth day, approximately one millilitre of venous blood was collected for hematological studies by nipping the tails of the mice. Collected blood was stored in EDTA treated plastic tubes at room temperature. 60 male white albino mice weighing on average 25 g were used. The hematological parameters determined using the Coulter Counter included white blood cells (WBC), haemoglobin (Hb), red blood cells (RBC), packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and mean cell volume (MCV). Mice that were observed to have haemoglobin and RBC levels of below 11 g/dL and $3.0 \times 10^{12}/L$, respectively were considered anemic.

Preparation of extracts for injection in mice

The appropriate doses of freeze-dried plant extracts were made by dissolving 125 mg (to deliver 50 mg/kg body weight), 250 mg (to deliver 100 mg/kg body weight), and 875 mg (to deliver 350 mg/kg body weight), in 10 ml physiological saline, respectively. 0.1 ml of the plant extract solution was orally administered to mice weighing on average 25 g.

Blood sampling was done by sterilizing the tail by wiping it with 10% alcohol and then nipping the tail with scissors. Blood was milked from the tail into EDTA treated vacutainer for the determination of white blood cell count, red blood cell count, hemoglobin, packed cell volume, mean cell hemoglobin, mean cell hemoglobin concentration and mean cell volume.

In vivo toxicity single dose studies

Fifteen (15) male albino mice weighing between 19-23 g were obtained from the Department of Zoological Sciences, Kenyatta University. The mice were divided into three different groups of five mice each. One group served as untreated control. The other two groups were treated with 1 g/kg body weight of each plant extract.

The 1 g/kg body weight dose was selected on the basis that the recommended doses for preliminary studies with plant extracts ranges between 50 and 300 mg/kg body weight of animal and toxicity is induced by three times the highest test dose (300 mg/kg body weight). The extracts were orally administered on a daily basis for a period of one month. During this period, mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behaviour and mortality. At the end of one month, they were sacrificed.

Determination of body weight

The body weight of each mouse was assessed during the acclimatization period, once before commencement of dosing, once weekly during the dosing period and on the day of sacrifice.

Absolute organ weight and histological assessment of *in vivo* toxicity

On the day of sacrifice, all the animals were euthanized using diethyl ether in a desiccator. Different organs including the heart, liver, spleen, and kidneys were carefully dissected out and weighed.

Preparation of blood samples for determination of hematological and biochemical parameters

After four weeks of experimentation, all animals were sacrificed and blood samples were drawn from the heart of each sacrificed animal. This blood was divided into two parts. One part was collected in plastic vacutainers treated with EDTA. Hematological parameters red blood cell count, packed cell volume, hemoglobin, mean cell hemoglobin concentration, mean cell hemoglobin, mean cell volume and white blood cell count were determined using a Coulter Counter machine. The other part was collected in plastic test tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20°C until required for biochemical parameter analysis.

Laboratory analysis

The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry Auto Analyser were alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine, creatinine kinase, amylase, gamma glutamyl transferase, and lactate dehydrogenase. All reagents for the machine were commercially prepared to fit the required volumes and concentrations. The reagents were in specific containers referred to as reagent cartridges. The reagent cartridges were bar coded for the identification by the machine. The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under the direct control of the instrument microprocessor. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.

Quality control (QC)

Precinorm U (normal upper) and precipath U (pathological upper) for all the parameters from Roche Diagnostics were the quality control materials used during the study period. Before use, a QC bottle was carefully opened and exactly 3 ml distilled water pipetted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then liquated into six cryovials and stored at -20°C. Calibrator used the same types of tubes and racks as samples. A refrigerated rack position in the machine improved the stability of on-board controls. The system performed controls automatically according to the specifications in the test definition.

Qualitative and quantitative determination of phytochemicals

Phytochemical analysis of the lyophilized plant extracts was conducted in accordance with standard procedures indicated in each test. The presence of saponins, phylobatannins, and cardiac glycosides were assessed as described by Krishnaiah et al. (2009) while that of

alkaloids, flavonoids, anthraquinones (free and bound), phenols, sterols and terpenoids and tannins were assessed as described by Houghton and Raman (1998) and Hossein and Hani (2002). The presence of tannins was confirmed by the method described by Banso (2009). The levels of alkaloids, flavanoids, saponins and tannins in the plant extract were estimated using the method described by Krishnaiah et al. (2009).

Preparation and determination of the mineral content of the plant extracts

At least three pellets weighing 300-1000 mg/cm² were prepared for analysis from the freeze dried plant sample using the press pellet machine and placed onto the sample tray. To enhance binding 25 mg of cellulose was mixed with the ground plant material. Total Reflection X-Ray Fluorescence (TXRF) system was used to determine the content of Manganese, Iron, Nickel, Copper, Zinc, Strontium, Potassium, Titanium, Chromium, Manganese and Calcium in the plant samples. The TXRF system analyser consists of an X-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source, Cd¹⁰⁹ (half-life, T_{1/2} = 453 days and activity = 10 mCi) are incident on the sample that emits the characteristic X-rays. These X-rays are detected by Si (Li) detector (EG&G Ortec, 30 mm² × 10mm sensitive volume, 25 µm Be window) with an energy resolution of 200 eV at 5.9 keV Mn K_α - line. The spectral data for analysis were

collected using personal computer based Canberra S-100 multi-channel analyzer (MCA). The acquisition time applied in the TXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software (QXAS, 1992) that is based on the fundamental parameters method (FPM). With this method, if the type and properties of all elements contained in a sample are known, then the intensity of each fluorescent X-ray is derived theoretically. By using this method, the composition of unknown sample is extrapolated by its fluorescence X-ray intensity of each element. The results are expressed in parts per million (ppm).

Data management and statistical analysis

Data generated from the study was entered in database designed using Microsoft Excel software, cleaned and exported into the SPSS software for statistical analysis. Data was expressed as Mean ± standard deviation (SD). The differences between the means of the various groups of animals in the efficacy study (normal, untreated anemic, anemic treated with 50, 100, and 350 mg/kg body weight of the aqueous extracts) was done using ANOVA and post ANOVA statistical test while the difference between the means of the two groups used in the toxicity study (normal, normal treated with 1 g/kg body weight of each plant) was done using student's T-test. The level of significance for all the analyses was set at a value of p < 0.05.

Blood parameter	Week	Normal	Anemic	Anemic (+ 50 mg/kg)	Anemic (+ 100 mg/kg)	Anemic (+ 350 mg/kg)
RBC (× 10 ¹² /L)	0	4.27 ± 0.50	1.73 ± 0.81 ^a	1.67 ± 0.76 ^b	1.27 ± 0.31 ^c	1.65 ± 0.57 ^d
	1	4.27 ± 0.31	2.07 ± 0.12 ^{ae}	2.10 ± 0.44 ^{be}	2.20 ± 0.20 ^{ce}	2.40 ± 0.20 ^{de}
	2	4.47 ± 0.31	2.27 ± 0.30 ^{ae}	3.23 ± 0.23 ^{bf}	3.13 ± 0.30 ^{cf}	2.60 ± 0.00 ^{de}
	3	4.27 ± 0.31	2.20 ± 0.35 ^{ae}	3.13 ± 0.12 ^{bf}	3.13 ± 0.12 ^{cf}	3.20 ± 0.40 ^{df}
	4	4.20 ± 0.20	2.60 ± 0 ± 20 ^{af}	3.40 ± 0.20 ^{bf}	3.40 ± 0.40 ^{cf}	3.30 ± 0.40 ^{df}
PCV (%)	0	40.53 ± 1.10	20.00 ± 3.49 ^a	20.07 ± 3.70 ^b	23.70 ± 7.86 ^c	15.20 ± 1.06 ^d
	1	41.27 ± 1.53	19.83 ± 1.63 ^a	22.93 ± 0.95 ^b	25.97 ± 1.04 ^c	25.53 ± 0.64 ^{de}
	2	41.50 ± 1.50	20.00 ± 1.78 ^a	25.70 ± 1.47 ^{be}	24.90 ± 1.74 ^c	26.50 ± 2.12 ^{de}
	3	41.47 ± 1.53	19.47 ± 1.40 ^a	25.27 ± 0.31 ^{be}	25.07 ± 0.12 ^c	27.53 ± 5.30 ^{de}
	4	41.50 ± 1.40	21.90 ± 1.00 ^a	28.90 ± 0.60 ^{bf}	28.10 ± 1.70 ^{ce}	26.50 ± 1.90 ^{de}
Hb (g/dL)	0	16.93 ± 0.83	8.30 ± 3.64 ^a	8.30 ± 3.03 ^b	8.85 ± 3.11 ^c	6.20 ± 0.00 ^d
	1	17.80 ± 0.20	8.53 ± 3.14 ^a	10.33 ± 0.12 ^b	9.67 ± 1.53 ^c	8.47 ± 1.55 ^{de}
	2	17.70 ± 0.16	8.40 ± 3.21 ^a	11.70 ± 0.80 ^{be}	11.30 ± 0.80 ^{ce}	10.30 ± 0.14 ^{df}
	3	17.80 ± 0.00	8.67 ± 3.21 ^a	11.13 ± 0.23 ^{be}	10.87 ± 0.31 ^c	12.40 ± 2.95 ^{dg}
	4	17.50 ± 0.50	11.60 ± 0.20 ^{ae}	14.00 ± 0.40 ^{bf}	14.70 ± 1.20 ^{cf}	14.10 ± 1.10 ^{dh}
MCHC (g/dL)	0	32.80 ± 1.44	28.87 ± 2.16	28.73 ± 2.20	27.75 ± 3.43 ^c	24.93 ± 0.61 ^d
	1	34.07 ± 0.23	27.20 ± 1.40 ^a	28.60 ± 1.93 ^b	27.07 ± 0.30 ^c	27.33 ± 0.42 ^d
	2	33.90 ± 0.23	27.27 ± 1.17 ^a	32.70 ± 0.23	30.60 ± 0.53 ^c	28.50 ± 0.99 ^d
	3	33.93 ± 0.31	26.87 ± 2.27 ^a	31.87 ± 0.23 ^b	31.60 ± 0.69 ^c	32.07 ± 0.42 ^{de}

	4	34.30 ± 0.40	28.10 ± 1.70 ^a	30.00 ± 0.90 ^b	29.70 ± 3.60 ^c	29.90 ± 5.20 ^d
	0	5.20 ± 0.40	6.30 ± 1.30	5.13 ± 1.15	4.90 ± 0.35	4.37 ± 0.40
	1	5.60 ± 0.20	6.30 ± 1.14	5.20 ± 0.00	4.80 ± 0.20	5.13 ± 0.12
WBC (× 10 ⁹ /L)	2	5.40 ± 0.20	6.00 ± 0.80	4.50 ± 0.11	4.30 ± 0.30	5.50 ± 0.14
	3	5.20 ± 0.60	5.20 ± 0.20	5.00 ± 0.40	4.80 ± 0.20	4.80 ± 0.20
	4	4.40 ± 0.30	5.10 ± 0.10	4.60 ± 0.80	4.60 ± 0.20	4.60 ± 0.20
	0	95.70 ± 8.60	133.30 ± 57.80	136.50 ± 53.10	144.60 ± 4.10	123.50 ± 23.00
	1	97.00 ± 5.80	96.00 ± 6.00	112.40 ± 23.50	118.90 ± 14.30	107.00 ± 11.40
MCV (fL)	2	92.80 ± 16.40	88.10 ± 13.40	79.60 ± 14.80	79.60 ± 16.60	101.90 ± 2.40
	3	97.10 ± 12.30	88.50 ± 11.80 ^a	80.70 ± 17.30	80.10 ± 16.90	86.00 ± 19.80
	4	98.20 ± 4.90	84.50 ± 3.30 ^a	84.20 ± 5.20 ^b	83.00 ± 5.60 ^c	81.40 ± 5.00 ^d
	0	39.90 ± 3.60	48.60 ± 2.30	52.00 ± 7.40 ^b	53.50 ± 2.40 ^c	50.80 ± 11.60
	1	41.80 ± 2.50	41.00 ± 13.90	50.50 ± 9.30 ^b	44.30 ± 9.60	35.70 ± 9.20
MCH (pg)	2	39.60 ± 4.90	37.00 ± 4.70	36.20 ± 4.40	36.10 ± 8.90	39.60 ± 6.70
	3	41.70 ± 12.40	39.40 ± 13.60	35.60 ± 8.60	34.70 ± 7.40	38.80 ± 11.70
	4	41.40 ± 1.30	44.90 ± 3.10	40.90 ± 0.80	43.30 ± 2.90	43.20 ± 2.20

Results are expressed as Mean ± standard deviation (SD) of five animals in each group. Differences between mean of the haematological parameter of the control mice and the anemic mice treated with each dose of the plant extracts and across the weeks were compared using the ANOVA and post ANOVA statistical test. ^ap < 0.05 is statistically significant when normal control animals are compared to anemic untreated animals; ^bp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 50 mg/kg body weight; ^cp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 100 mg/kg body weight; ^dp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 350 mg/kg body weight; ^eg^hp < 0.05 represents significant difference within and among the measured parameters for the four weeks of the study period.

Table 1: Effects of oral administration of varying doses of aqueous stem bark extracts of *Z. usambarensis* in mice for four weeks on hematological parameters.

Results

Table 1 shows the effects of oral administration of aqueous stem bark extracts of *Z. usambarensis* in mice for four weeks on hematological parameters. Results show that continued daily administration of the three doses of the aqueous stem bark extracts of *Z. usambarensis* in mice for four weeks significantly increased the reduced levels of RBC, PCV and Hb but not to normal values; however, this extract did not significantly affect the levels of MCV, MCH, MCHC and WBC; as depicted in Table 2, continued daily administration of the three doses of the aqueous stem bark extract of *E. abyssinica* in mice for four weeks significantly increased the reduced levels of RBC, PCV and Hb and did not significantly affect the levels of MCV, MCH, MCHC and WBC. However, the 100 mg/kg body weight dose returned the measured hematological parameters to normal values in the third week and remained so even in the fourth week.

Effects of oral administration of high doses of aqueous extracts of *Z. usambarensis* and *E. abyssinica* in mice for one month on body and organ weights

As depicted in Tables 3 and 4, oral administration of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* in mice at a dose of 1 g/kg body weight daily for four weeks had no significant effect on both the body and organ weight.

Effects of oral administration of high doses of aqueous extracts of *Z. usambarensis* and *E. abyssinica* in mice for one month on the measured biochemical and hematological parameters

As depicted in Table 5, oral administration of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* in mice at a dose of 1 g/kg body weight daily for four weeks significantly raised the levels of urea and creatinine and activities of alkaline phosphatase, lactate dehydrogenase, alpha-amylase and gamma-glutamyltransferase. As shown in Table 6, oral administration of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* in mice at a dose of 1 g/kg body weight daily for four weeks had no significant effect all the measured hematological parameters.

Phytochemical composition of aqueous extracts of *Z. usambarensis* and *E. abyssinica*

Phytochemical screening of aqueous extracts of *Z. usambarensis* and *E. abyssinica* show that alkaloids and flavonoids were present in both *Z. usambarensis* and *E. abyssinica*; bound and free anthraquinones were present in *Z. usambarensis* and absent in *E. abyssinica*; tannins were absent in *Z. usambarensis* and *E. abyssinica*; sterols, terpenoids and phylobatannins were only present in *Z. usambarensis*; *E. abyssinica* contained cardiac glycosides.

E. abyssinica extract had an alkaloid concentration of 95(±10) mg/100 g with a yield of 0.19% (w/w) and a flavanoid concentration of 150(±20) mg/100 g with a yield of 0.15% (w/w) while *Z. usambarensis* had an alkaloid concentration of 67(±10) mg/100 g with a yield of 0.13% (w/w) and a flavanoid concentration of 87 mg/100 g with a yield of 0.087% (w/w).

Mineral composition of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica*

Table 7 shows the mineral content of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* and the mineral content in 1 g plant extracts orally administered to each mouse per day (µg/day) for 30 days. Results show that the aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* contain the macro minerals potassium (K), and calcium (Ca) and micro minerals chromium (Cr), manganese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn) and the toxic heavy metals arsenic (As), nickel (Ni) and lead (Pb) at varying concentrations. Table 7 also shows that administration of 1g of the aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* per kilogram body weight of mouse daily for 30 days provided more than

the recommended daily requirements for the macrominerals K and Ca and microminerals Cr and Mn. This daily requirement was also exceeded for Fe in the aqueous stem bark extracts of *E. abyssinica*. All the aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* orally administered at 1 g per kilogram body weight provided Zn at levels below the recommended daily requirements. This daily requirement provision was also below for Fe in the aqueous stem bark extract of *Z. usambarensis*.

Discussion

Sub-chronic intoxication of mice with 5 mg per kilogram body weight Phenylhydrazine (PHZ) for eight days resulted in anemia characterized by decreased red blood cell count, hemoglobin and packed cell volume and normal mean cell hemoglobin, mean cell hemoglobin concentration, mean cell volume and white blood cell count. Similar results were reported in rats administered with Phenylhydrazine to induce anemia [20]. Administration of PHZ to rats has been reported to result in the production of both aryl and hydroxyl radicals which produce oxidative stress on the red cell membrane resulting in haemolysis by lipid peroxidation [21,22].

Blood parameter	Week	Normal	Anemic	Anemic + 50 mg/kg	Anemic + 100 mg/kg	Anemic + 350 mg/kg
RBC (× 10 ¹² /L)	0	4.27 ± 0.50	1.73 ± 0.81 ^a	1.27 ± 0.50 ^b	2.30 ± 0.26 ^c	2.10 ± 0.58 ^d
	1	4.27 ± 0.31	2.07 ± 0.12 ^a	1.80 ± 0.40 ^{be}	2.73 ± 0.12 ^c	2.93 ± 0.22 ^{de}
	2	4.47 ± 0.31	2.27 ± 0.30 ^{ae}	3.60 ± 0.20 ^{bf}	3.90 ± 0.10 ^{ce}	3.60 ± 0.10 ^{df}
	3	4.27 ± 0.31	2.20 ± 0.35 ^{ae}	3.33 ± 0.83 ^{bf}	4.30 ± 0.31 ^e	3.93 ± 0.76 ^f
	4	4.20 ± 0.20	2.60 ± 0 ± 20 ^{ae}	3.50 ± 0.80 ^{bf}	4.30 ± 0.10 ^e	3.90 ± 0.10 ^f
PCV (%)	0	40.53 ± 1.10	20.00 ± 3.49 ^a	20.73 ± 5.40 ^b	22.03 ± 1.42	22.50 ± 0.93
	1	41.27 ± 1.53	19.83 ± 1.63 ^a	23.93 ± 1.22 ^b	23.87 ± 0.76	23.55 ± 0.64
	2	41.50 ± 1.5	20.00 ± 1.78 ^a	33.50 ± 1.25 ^{be}	34.90 ± 0.94 ^e	29.60 ± 0.94 ^e
	3	41.47 ± 1.53	19.47 ± 1.40 ^a	34.00 ± 4.61 ^{be}	42.60 ± 3.08 ^f	34.47 ± 5.08 ^e
	4	41.50 ± 1.40	21.90 ± 1.00 ^a	34.60 ± 5.10 ^{be}	42.00 ± 2.10 ^f	39.70 ± 1.00 ^f
Hb (g/dL)	0	16.93 ± 0.83	8.30 ± 3.64 ^a	6.67 ± 2.66 ^b	10.27 ± 0.83 ^c	8.28 ± 2.89 ^d
	1	17.80 ± 0.20	8.53 ± 3.14 ^a	7.13 ± 1.97 ^b	10.47 ± 0.42 ^c	10.85 ± 0.68 ^{de}
	2	17.70 ± 0.16	8.40 ± 3.21 ^a	14.40 ± 0.68 ^{be}	15.00 ± 0.47 ^{ce}	13.25 ± 0.47 ^{df}
	3	17.80 ± 0.00	8.67 ± 3.21 ^a	14.00 ± 3.29 ^{be}	18.00 ± 1.66 ^f	16.40 ± 1.39 ^g
	4	17.50 ± 0.50	11.60 ± 0.20 ^a	14.90 ± 1.30 ^{be}	17.50 ± 0.90 ^f	16.60 ± 0.40 ^g
MCHC (g/dL)	0	32.80 ± 1.44	28.87 ± 2.16	27.20 ± 3.22	29.53 ± 2.67	30.05 ± 1.00
	1	34.07 ± 0.23	27.20 ± 1.40	27.87 ± 3.06	29.40 ± 1.22	31.00 ± 1.05
	2	33.90 ± 0.23	27.27 ± 1.17	30.20 ± 0.72	30.50 ± 0.23	31.12 ± 0.23
	3	33.93 ± 0.31	26.87 ± 2.27 ^a	31.40 ± 1.22	34.06 ± 1.03	32.47 ± 1.17
	4	34.30 ± 0.40	28.10 ± 1.70 ^a	32.20 ± 1.10	33.10 ± 0.20	31.50 ± 1.40
	0	5.20 ± 0.40	6.30 ± 1.30	5.20 ± 1.06	5.40 ± 0.60	5.43 ± 0.13
	1	5.60 ± 0.20	6.30 ± 1.14	4.82 ± 0.64	5.33 ± 0.64	5.00 ± 0.37

WBC (× 10 ⁹ /L)	2	5.40 ± 0.20	6.00 ± 0.80	4.70 ± 0.30	4.26 ± 0.23	4.63 ± 0.23
	3	5.20 ± 0.60	5.20 ± 0.20	5.00 ± 0.35	4.93 ± 0.42	4.73 ± 0.12
	4	4.40 ± 0.30	5.10 ± 0.10	4.60 ± 0.20	4.70 ± 0.40	4.50 ± 0.30
	0	95.70 ± 8.60	133.3 ± 57.8	171.90 ± 41.00	96.30 ± 7.40	113.30 ± 31.70
	1	97.00 ± 5.80	96.00 ± 6.00	138.00 ± 36.00	87.30 ± 1.00	80.80 ± 6.10
MCV (fL)	2	92.80 ± 16.40	88.10 ± 13.40	93.10 ± 15.60	89.50 ± 12.30	82.20 ± 14.80
	3	97.10 ± 12.30	88.50 ± 11.80	102.10 ± 16.90	99.11 ± 18.90	87.70 ± 13.00
	4	98.20 ± 4.90	84.50 ± 3.30	101.50 ± 12.70	97.80 ± 6.40	101.80 ± 1.20
	0	39.90 ± 3.60	48.60 ± 2.30 ^a	52.80 ± 2.50 ^b	45.20 ± 8.20	38.70 ± 7.80
	1	41.80 ± 2.50	41.00 ± 13.90	41.00 ± 13.20	38.40 ± 2.50	37.10 ± 1.40
MCH (pg)	2	39.60 ± 4.90	37.00 ± 4.70	40.00 ± 3.80	38.50 ± 5.80	36.80 ± 4.80
	3	41.70 ± 12.4	39.40 ± 13.60	42.00 ± 13.80	41.91 ± 6.70	41.70 ± 13.90
	4	41.40 ± 1.30	44.90 ± 3.10	44.00 ± 7.30	40.70 ± 2.80	42.40 ± 0.50

Results are expressed as Mean ± standard deviation (SD) of five animals in each group. Differences between mean of the haematological parameter of the control mice and the anemic mice treated with each dose of the plant extracts and across the weeks were compared using the ANOVA and post ANOVA statistical test. ^ap < 0.05 is statistically significant when normal control animals are compared to anemic untreated animals; ^bp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 50 mg/kg body weight; ^cp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 100 mg/kg body weight; ^dp < 0.05 is statistically significant when normal control animals are compared to anemic animals treated with 350 mg/kg body weight; ^{e,f,g,h}p < 0.05 represents significant difference within and among the measured parameters for the four weeks of the study period.

Table 2: Effects of oral administration of varying doses of aqueous stem bark extracts of *E. abyssinica* in mice for four weeks on hematological parameters.

Treatment	Week				
	0	1	2	3	4
Normal	22.30 ± 1.60	23.08 ± 1.50	24.14 ± 1.10	25.12 ± 0.97	26.34 ± 0.94
<i>Z. usambarensis</i> (1 g/kg body weight)	21.38 ± 1.36	22.50 ± 1.40	23.32 ± 1.31	24.28 ± 1.14	25.58 ± 1.11
<i>E. abyssinica</i> (1 g/kg body weight)	22.36 ± 0.90	23.08 ± 0.85	22.02 ± 1.06	24.7 ± 0.92	25.64 ± 0.84

Results are expressed as Mean ± standard deviation (SD) for five animals per group. Differences between mean body weight (in g) of the control mice and mice treated with 1 g/kg of each of the plant extracts was compared using the Student's t-test. p < 0.05 was considered statistically significant.

Table 3: The effects of oral administration of high doses of aqueous plant extracts of *Z. usambarensis* and *E. abyssinica* in mice for one month on body weights.

Ferrali et al. (1997) [23] reported increased reticulocytosis, methaemoglobinemia and haemocatheresis in PHZ intoxicated rats. The PHZ induced anemia was restored to normal by oral administration of aqueous stem bark extracts of *E. abyssinica* at a dose of 100 mg per kilogram body weight daily within 21 days.

A similar dose of *Z. usambarensis* extracts orally administered daily for 21 days could not restore the PHZ induced anemic state to normal.

This observation could be explained by the lower levels of alkaloids and flavonoids and high manganese: iron, iron: zinc and copper: zinc ratio in *Z. usambarensis* extracts compared to *E. abyssinica* extracts which has higher iron: zinc and iron: manganese ratio.

Copper and iron have a synergistic interaction which promotes hematopoiesis while iron-manganese, copper-zinc, and iron-zinc interactions are all antagonistic. Other antagonistic interactions participating in *Z. usambarensis* which could impair iron absorption are selenium-cadmium, selenium-mercury, selenium-arsenic, calcium-iron, calcium-zinc, molybdenum-iron, and zinc-cadmium; however, the levels of selenium, mercury, and molybdenum were not estimated.

Organ	Heart	Liver	Spleen	Kidney
Normal	0.14 ± 0.02	1.81 ± 0.04	0.27 ± 0.01	0.26 ± 0.01
<i>Z. usambarensis</i> (1 g/kg body weight)	0.14 ± 0.01	1.78 ± 0.04	0.25 ± 0.00	0.29 ± 0.01
<i>E. abyssinica</i> (1 g/kg body weight)	0.16 ± 0.01	1.94 ± 0.05	0.28 ± 0.00	0.28 ± 0.01

Results are expressed as Mean ± standard deviation (SD) for five animals per group. Differences between mean of the weight (in g) of the individual organs of the control mice and mice treated with 1 g/kg of each of the plant extracts was compared using the Student's t-test. p < 0.05 was considered statistically significant.

Table 4: The effects of oral administration of high doses of aqueous plant extracts of *Z. usambarensis* and *E. abyssinica* in mice for one month on organ weights.

In antagonistic interactions an excess of one mineral reduces and/or affects the presence of the other. This phenomenon takes place when competing ions possess the same, or very similar, electron configuration. In synergistic interactions, the uptake of one mineral promotes the uptake of the other. The high levels of calcium in *E. abyssinica* extracts also inhibit zinc absorption. The speedy and progressive recovery of anemic mice on treatment with *E. abyssinica* extracts could be due to increased erythropoiesis. This restored anemic situation was not altered by using higher doses of *E. abyssinica* extracts (0.35-1 g/kg body weight) indicating the mice control system over polycythaemia. Under normal conditions, the body generates new red

blood cells to replace the lost ones but this takes longer as observed in the untreated anemic mice. *E. abyssinica* could also be containing higher levels of folic acid, vitamin B₁₂, vitamin E, vitamin C and vitamin B₆ than *Z. usambarensis* which may also account for the faster reversal of PHZ induced anemia; however, the levels of these vitamins were not estimated in this study. All these vitamins together with copper, iron and optimal balance of other minerals such as cobalt are required for optimal erythropoiesis. These vitamins have been reported in *Brillantaisia nitens* which exhibits hematinic activity [24]. Deficiency of vitamin B₁₂ and folic acid causes macrocytic, megaloblastic and pernicious anaemia [25].

Treatment	Biochemical Parameter								
	UREA	CREAT	AST	ALT	ALP	GGT	LDH	CK	AMY
	(mmol/L)	(μmol/L)	(U/L)	(U/L)	(U/L)	(U/L)	(U/L)	(U/L)	(U/L)
Normal	8.7 ± 0.8	27.3 ± 5.8	1243.0 ± 342.9	164.7 ± 22.5	16.0 ± 6.6	6.0 ± 2.0	6534.0 ± 2261.9	732.3 ± 119.5	1767.3 ± 520.7
<i>Z. usambarensis</i> 1 g/kg body weight	10.3 ± 0.1	32.3 ± 2.1	1414.0 ± 63.2	191.3 ± 41.7	34.7 ± 5.0^a	10.0 ± 2.0^a	10489.3 ± 2793.8^a	904.3 ± 10.2	2686.3 ± 336.2^a
<i>E. abyssinica</i> 1 g/kg body weight	10.6 ± 0.5	39.0 ± 6.6^a	1206.0 ± 253.4	189.3 ± 23.9	45.0 ± 10.6^a	10.0 ± 4.4^a	11045.3 ± 1823.9^a	898.0 ± 38.9	2320.0 ± 217.6^a

Results are expressed as Mean ± standard deviation (SD) of five animals in each group. Differences between mean of the measured biochemical parameters of the control mice and mice treated with 1 g/kg of each of the plant extracts was compared using the Student's t-test. p < 0.05 was considered statistically significant.

Table 5: The effects of oral administration of high doses of aqueous plant extracts of *Z. usambarensis* and *E. abyssinica* in mice for one month on the biochemical parameters.

Treatment	RBC (× 10 ¹² /L)	PCV (%)	Hb (g/dL)	MCH (pg)	MCHC (g/dL)	MCV (fL)	WBC (× 10 ⁹ /L)
Normal	4.40 ± 0.49	42.26 ± 1.19	16.96 ± 1.37	38.60 ± 1.70	31.38 ± 2.10	96.60 ± 8.40	5.22 ± 0.31
<i>Z. usambarensis</i> (1 g/kg body weight)	4.28 ± 0.28	41.98 ± 0.69	16.66 ± 0.58	38.20 ± 1.70	33.40 ± 0.90	97.30 ± 6.80	5.22 ± 0.32
<i>E. abyssinica</i> (1 g/kg body weight)	4.38 ± 0.34	42.42 ± 0.50	16.68 ± 0.99	39.00 ± 1.40	33.30 ± 0.58	98.40 ± 5.40	4.98 ± 0.19

Results are expressed as Mean ± standard deviation (SD) of five animals in each group. Differences between mean of the measured haematological parameters of the control mice and mice treated with 1 g/kg of each of the plant extracts was compared using the Student's t-test. p < 0.05 was considered statistically significant.

Table 6: Effect of oral administration of high oral doses of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* in mice daily for one month on some end point hematological parameters.

Folic acid relieves symptoms in patients who have nutritional macrocytic anaemia, macrocytic anaemia of pellagra, megaloblastic anaemia of pregnancy, and megaloblastic anaemia of infancy [26,27]. Deficiency of iron in humans and animals leads to iron deficiency anaemia. Iron deficiency causes anaemia in children of 6 months to 2 years [28], pregnant women and menstruating women [29].

Anemia has also been reported in vitamin B₁₂ and folate deficiency and in rats infected with *Trypanosoma brucei* since these three induce iron deficiency anemia [30-33]. PHZ induced anemia was also restored in rats orally administered with aqueous extracts of *Hibiscus cannabinus* at a dose of 400 mg per kilogram body weight daily for three weeks [20]. The raised levels of urea and creatinine and activities of alkaline phosphatase, lactate dehydrogenase, gamma-glutamyltransferase all of which are indicators of kidney injury [31,34]

and α-amylase a biomarker of pancreatic injury in mice administered with high doses of these plant extracts daily for thirty days (1 g/kg body weight) compared to those of normal control mice could be due to the observed presence of lead, nickel, and arsenic which are known to cause kidney damage [31,34] and excess calcium, potassium, iron and manganese in addition to the presence of toxic alkaloids, cardiac glycosides and flavonoids in the extracts.

Excess blood calcium could be toxic since it results in kidney stones formation, hypercalcemia and renal insufficiency with or without alkalosis, and reduced absorption of iron, zinc, magnesium and phosphorus. Excess blood potassium results in hyperkalemia due to either a shift of potassium from cells to the ECF or excess potassium retention caused by trauma and infection, metabolic acidosis, and chronic renal failure. A clinical consequence of potassium excess is

cardiac arrest. Potassium and sodium interactions determine the risk of coronary heart disease and stroke. Potassium interacts with calcium to regulate the acid-base balance and ameliorates the effects of sodium on calcium deficiency. Excess iron intake does not result in iron overload because of the effective regulation of iron absorption. However, excess blood iron causes cellular and tissue injury; increases the risk of bacterial infection, neoplasia, arthropathy, cardiomyopathy, and endocrine dysfunction. Excess manganese inhibits iron absorption; these micronutrients exhibit antagonistic interactions to each other [35].

Mineral	Mineral composition of the stem bark extracts (mg/100 g)		Daily administered mineral (µg/day)		RDA for mouse per day (µg/day)
	Z. usambarensis	E. abyssinica	Z. usambarensis	E. abyssinica	
K	1860.00 ± 110.00	1066.00 ± 168.00	454	270	75
Ca	37.75 ± 1.85	3260.00 ± 262.00	9.4	820	1.3
Ti	8.40 ± 0.29	18.90 ± 11.60	2.1	4.8	0.015
Cr	12.550 ± 2.050	2.093 ± 1.011	3.1	0.525	0.01
Mn	360.50 ± 37.50	9.57 ± 0.69	90	2.4	0.82
Fe	2.02 ± 0.17	175.70 ± 13.60	0.5	44	10.71
Cu	4.055 ± 0.285	0.800 ± 0.341	1	0.2	0.32
Zn	0.186 ± 0.179	0.832 ± 0.323	0.048	0.2	6.79
Ni	0.435 ± 0.029	0.197 ± 0.178	0.1	0.05	-
As	0.358 ± 0.055	0.321 ± 0.059	0.088	0.08	-
Pb	1.665 ± 0.235	0.205 ± 0.071	0.43	0.05	-

Results are expressed as Mean ± standard deviation (SD) for three replicates for each plant extract.

Table 7: Minerals composition of the aqueous stem bark extract of *Z. usambarensis* and *E. abyssinica* (mg/100 g) and the quantity of each mineral in 1 g plant extracts per kg body weight orally administered to each mouse per day (µg/day).

This observed toxicity could also be associated with the presence of phytonutrients which have both positive and negative effects to human and livestock. Phytonutrients such as pyrrolizidine alkaloids and hydrolysable tannins are toxic [36]. Acute poisoning by pyrrolizidine alkaloids in humans is characterised by haemorrhagic necrosis, hepatomegaly and ascites; death is caused by liver failure due to necrosis and liver dysfunctions. Sub-acute toxicity is characterised by hepatomegaly and recurrent ascites; endothelial proliferation and medial hypertrophy leading to an occlusion of hepatic veins, resulting in the veno-occlusive disease (VOD) in which the veins are narrowed.

The VOD causes centrilobular congestion, necrosis, fibrosis and liver cirrhosis, the end-stage of chronic pyrrolizidine alkaloid intoxication [36]. However, liver toxicity based on alterations in the measured biochemical parameters was not demonstrated in the present study.

Hydrolysable tannin toxicity is associated with haemorrhagic gastroenteritis, necrosis of the liver and kidney damage with proximal tubular necrosis [37]. Tannin toxicity is also characterized by anorexia, depression, ruminal atony, hepatic and renal failure, ulcers along the digestive tract and severe gastroenteritis [37]. While tannins could lead to kidney damage, they were absent from the two plant extracts. Again since flavonoid toxicity to animals is very low (in rats the LD₅₀ is 2-10 g for most flavonoids) [38], their presence in these plant extracts may also not be associated with the observed toxicity to the kidneys and the pancreas of the treated mice. The toxicity of *E. abyssinica* extracts on the kidney and pancreas of the treated mice could also be due to the presence of cardiac glycosides. The presence of cardiac glycosides may account for the paralysis and respiration failure reported to be associated with the injection of seed extracts of *E. abyssinica* into the bloodstream as an anaesthetic [9,14].

Conclusion

In conclusion, the study has confirmed *in vivo* haematinic activity of aqueous stem bark extracts of *Z. usambarensis* and *E. abyssinica* used in management of anemia in Makueni district. Both the observed haematinic activity and the slight toxicities observed in these two plants could be attributed to some of the phytochemicals and toxic minerals present in the plant extracts. The study recommends continued use of *Z. usambarensis* and *E. abyssinica* in the management of anaemia.

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