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Research Article
Therapeutic Effects of a Polyherbal Formula on Some Coagulation Indices in Haemoglobinopathy

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Abstract

Background and Objective: Hypercoagulability, inflammation, cardiovascular problems and stroke are common complications of haemoglobinopathies and a leading cause for hospital admission among patients. The influence of a polyherbal formula on endothelial dysfunction, coagulation indices and inflammation was investigated in 2-butoxyethanol-induced experimental model of haemoglobinopathy in adult rabbits. **Materials and Methods:** A total number of 20 healthy rabbits were randomly selected for this study and divided into groups. Twelve rabbits were administered 2.5 mL kg^{-1} of 2-butoxyethanol *per os* (p.o) and 1 mL kg^{-1} of 100 mg mL^{-1} polyherbal formula for 15 days, respectively pre- and post-2-butoxyethanol administration while the remaining six (6) were grouped as non-exposed/control. The levels of coagulation indices, inflammation marker and platelets were then evaluated. Tissue histology was used to assess the expression of microvascular occlusion and ischaemia. **Results:** Polyherbal formulation treatment significantly ($p<0.05$) reduced circulating C-reactive protein level as well as the platelet dyscrasias and associated endothelial dysfunction by reducing the levels of coagulation indices. There was also down-regulation of the expression of tissue necrosis in the liver and spleen of rabbits treated with 2-butoxyethanol as a result of Polyherbal treatment. **Conclusion:** Polyherbal formulation was found not to have any metabolic toxicity and was able to prevent 2-butoxyethanol-induced hypercoagulability and circulating thrombose as well as endothelial and inflammatory perturbation in rabbits when administered for 15 days before and after exposure to the 2-butoxyethanol induced haemoglobinopathy complications.

Key words: 2-butoxyethanol, anti-thrombotic, anti-inflammatory, haemoglobinopathy, polyherbal formulation

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Haemoglobinopathies are a group of dissimilar disorders instigated by interruption of the globin gene expression with approximately 7% of the worldwide population being carriers. Haemoglobinopathies represent the most common monogenic diseases and one of the world's major health problems occurring at high frequencies particularly in the tropical and sub-tropical areas where malaria is said to be widespread[1,2]. The HbS which causes Sickle Cell Disease (SCD), HbC and HbE are the most common structural haemoglobin variants sub-class of haemoglobinopathy and most prevalent monogenic disease worldwide with their incidence linked to the prevalence of malaria and seen mainly in the tropical regions such as Nigeria and other parts of West Africa[3]. Haemoglobinopathies are characterized by varied clinical heterogeneity that can be linked to two main pathogenic developments which are chronic haemolysis and high viscosity or obstruction within the blood vessels. Amplified adhesiveness of the white blood cells, platelets as well as other sickled cells to the endothelium of blood vessels is a leading cause for hospital admission among patients with haemoglobinopathies[4,5]. Prominent complications of most haemoglobinopathies commonly the SCD include increased risk of cardiovascular complications and hypercoagulability with a higher chance of developing conditions like pulmonary hypertension, stroke and even complications that may arise from pregnancy, which may occur as a result of thrombotic vascular occlusion (thromboembolism), the underlying mechanism of which remains under study[6,7]. Thromboembolic complications have also been observed to be common in thalassemia patients.

The management of the vaso-occlusive painful episodes, hypercoagulability and thromboembolic complications associated with haemoglobinopathies is a challenge to the physician and the most commonly prescribed medication for the treatment of this condition is hydroxyurea and opioid analgesics since there is no known cure for haemoglobinopathies[8]. Although the goal of these drugs have been to decrease the vaso-occlusive painful episodes, hypercoagulability, thromboembolic complications, joint inflammation and to relieve pain, these drugs are however known to produce various side effects including resultant myelosuppressive effects on leucocytes and platelets, immunodeficiency and male infertility in some patients[9]. The hydroxyurea is a chemotherapeutic agent that is both cytotoxic and genotoxic while the opioid analgesics are addictive and hepatotoxic[10]. Reducing these numerous side effects should be considered while designing improved therapeutics for haemoglobinopathies, besides enhancing medicinal effectiveness accordingly.

Hence, herbal treatments that possess anti-inflammatory, anti-thrombotic and antioxidant properties are being increasingly recognized as alternate approach in preventing haemoglobinopathy complications. Polyherbal formulations (PHFs) are now renowned for expressing high effectiveness in a vast number of disease conditions. Therapeutic effect of polyherbal medicines are exerted due to the concerted efforts of different phytoconstituents in the herbs which effects are further potentiated when compatibly formulated together in the solution[11]. The Polyherbal formulation consists of five plants which were, *Hunteria umbellate*, *Calliandra portoricensis*, *Kigelia africana*, *Lagenaria breviflora* and *Nauclea latifolia*. Although, each of these plants has been reported to exert antidiabetic, analgesic, antispasmodic, antipyretic, immuno-stimulatory[12,13], antihyperlipidemic, anti-thrombotic[14], anti-inflammatory, anxiolytic and antioxidant[15] activities, the active phytochemical constituents of individual plants are however insufficient to achieve the desirable therapeutic effects but when the multiple herbs are combined in a particular ratio, it will give a better therapeutic effect and reduce the toxicity. This study, therefore, investigated the medicinal activities of the polyherbal formulation on the coagulation indices and a selected inflammatory marker in 2-butoxyethanol (2-BE)-induced experimental model of haemoglobinopathy in adult rabbits.

MATERIALS AND METHODS

Plants materials: The five medicinal plants *Hunteria umbellate*, *Calliandra portoricensis*, *Kigelia africana*, *Lagenaria breviflora* and *Nauclea latifolia*, which were used for the preparation of the polyherbal formulation were purchased from local vendors at Kajola farm settlement, Ejigbo Local Government Area of Osun State. The plants with their selected parts were identified at the Botany Department, Obafemi Awolowo University and later authenticated taxonomically at the Department of Pharmacognosy, Obafemi Awolowo University, Ile-Ife, Osun State.

Preparation of the polyherbal formulation: The fresh stem barks and leaves of the various herbs used in the polyherbal formulation were washed properly, cut into small pieces and dried under shade. Each individual constituent was ground

into tiny pieces and 20 g of each plant was extracted separately by soaking in 200 mL of methanol and allowed to stand overnight before being filtered. The residues were re-suspended in an equal volume of methanol for 48 h before being filtered again. The whole extract of individual plants was collected in conical flasks, filtered and the solvents were evaporated to dryness under reduced pressure. The dried extracts from the various herbs were reconstituted with distilled water before administration.

Administration of 2-Butoxyethanol: The 2-butoxyethanol (2-BE) solution used was purchased from Sigma Aldrich GmBH (Steinheim, Germany) and diluted in water for oral administration at a concentration of 0.18 mL of the 2-BE to 2.32 mLs of water to make up 2.5 mLs kg^{-1} of 2-BE. Precisely 2.5 mLs kg^{-1} of 2-BE *p.o* was administered using oral gavage every day for the induction period, respectively. The rabbits administered with 2-BE were allowed to grow under observation and tested periodically until the haemoglobinopathic features of interest developed in significant numbers of them which were separated for further experimental treatment protocol.

Preliminary phytochemical analysis: Phytochemical screening of the crude extract of the leaves was carried out to ascertain the qualitative chemical composition of the plant using commonly employed procedures described by Harborne[16] and Adedapo *et al.*[17] to identify the major constituents.

Animals and experimental design: A total number of 20 mature New Zealand rabbits, weighing about 1.5±0.5 kg were used for the experiment. Animals were housed in solid-bottomed aluminum cages, subjected to standard 12 h light and dark cycle and were fed with commercial pellets and water *ad libitum*. The design and conducts of the experiments were in accordance with the Medical Research Ethical Committee guidelines for Clinical and Experimental Researches in Afe Babalola University, Ado-Ekiti, all instructions, principles of laboratory animal care and protocols regarding treatment during the experiments were conducted in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals. The rabbits were allowed to acclimatize to their environment for 2 weeks before the commencement of the experiments and were examined to be free of wounds, swellings and infections. The rabbits were randomly distributed into six groups of 3 animals per group according to the treatment received for 15 days of the study as follows and represented by Table 1:

A (n = 3): Feed alone+Water (Negative/Naïve control)
B (n = 3): 2-BE+feed (Haemoglobinopathy-induced/Positive control)
C (n = 3): Polyherbal treatment+2-BE (Prophylactic group)
D (n = 3): Post 2-days 2-BE Polyherbal treatment (Therapeutic Group 1)
E (n = 3): Post 3-days 2-BE+Polherbal treatment (Therapeutic Group 2)
F (n = 3): Feed+Polyherbal treatment (Adverse control Group)

Acute toxicity study: The acute oral toxicity study of the polyherbal formula was determined according to the method of Sawadogo *et al.*[18].

Polyherbal treatment: Animals were randomly distributed into six groups of three animals each (n = 3). Group A served as Negative control (2.5 mL kg^{-1} of distilled water *p.o* daily) and received commercial feed and water only, Group B served as Positive Control and was administered 2-BE (2.5 mL kg^{-1}) *p.o.* daily for 2 days, Group C served as Prophylactic and received Polyherbal treatment *p.o.* for 15 days followed by induction with 2-BE for 2 days. Groups D and E animals were administered 2-BE solution (2.5 mL kg^{-1}) *p.o.* daily for 2 and 3 days, respectively before being treated with the polyherbal formulation *p.o.* daily for 15 days, respectively.

Table 1: Experimental protocol

Groups	Treatments	Inference
A (n = 3)	Feed alone+water	Negative control
B (n = 3)	*2-BE+Feed	Haemoglobinopathy-induced/Positive control
C (n = 3)	Polyherbal+*2-BE	Prophylactic group
D (n = 3)	2-days post *2-BE+Polyherbal	Therapeutic group 1
E (n = 3)	3-days post *2-BE+Polyherbal	Therapeutic group 2
F (n = 3)	Feed+Polyherbal	Adverse group

*2-Butoxyethanol (2-BE) effects were investigated in each of the treatment groups

Group F received only the polyherbal formulation for 15 days *p.o.* daily. The prepared extract was administered by gavage once daily for 15 consecutive days with the aid of oral cannula and the dose administered to the appropriate experimental rabbits was 1 mL kg^{-1} of 100 mg mL^{-1} polyherbal formula for 15 days, respectively.

Sample collection: The blood specimens were collected from the inferior vena cava by the use of 5 mL syringe, dispensed into Tri-sodium citrate anticoagulant bottles and ethylene diamine tetra-acetic acid (EDTA) vials, mixed gently and appropriately labelled. The tests were carried out as soon as possible after sample collection. The experimental animals were sacrificed after overnight fasting upon conclusion of the experiment under light ether anesthesia by cervical dislocation.

Analytical methods

Determination of platelet Indices: Platelet counts were evaluated by flow cytometry (direct current method) using the Automated Haematological Analyzer, Sysmex, KXN21 (Japan) with the aid of suitable cell packs as described by Akinbo *et al.*[19]. Platelet manual count was also conducted to complement the automation estimation result using Neubauer counting chamber technique as previously described by Cheesbrough[20].

Clotting profile: Blood coagulation indices, PT and aPTT were measured by DiaPlastin and DiaClin kits (DiaMed GmbH, Switzerland) according to the instructions of the manufacturer[20,21].

Expression of C-Reactive protein (CRP): The level of expression of peripheral C-reactive protein was assessed using the Roche Cobas Immunoturbidimetric Analyzer Model C111, Particle-Enhanced Immunoturbidimetric technique[22]. In brief, the latex particles of uniform size are coated with monoclonal antibodies (F(ab')2 fragments) to the CRP epitope. The antigen/antibody complexes produced by the addition of samples containing CRP lead to an increase in the turbidity of the test reactants. The change of absorbance with time is dependent on the concentration of CRP epitopes in the sample. The precipitate is then determined turbidimetrically.

Histopathological studies: After blood collection, the animals were sacrificed and the organs from the various treatment groups were harvested and fixed immediately in 10% formol-saline. Tissues were then sectioned and finally stained with Haematoxylin and Eosin to examine the histopathological changes during the experimental period[23,24].

Statistical analysis: Data were expressed as Mean±SEM of a replicate in each group. One-way analysis of variance (ANOVA) was employed to determine variance among the study groups, while least square difference test was employed to determine the differences between means of collected data. Differences were considered significant at the levels of $p = 0.05$.

RESULTS

Phytochemical analysis: Preliminary phytochemical profiling of the various plant extracts in the polyherbal formula revealed the presence of desired phyto constituents in each extract and results were shown in the Table 2.

Acute toxicity study: Oral administration of graded doses (100, 400, 800, 1600 and 3200 mg kg^{-1}) of the polyherbal formulation did not produce any significant changes in behaviour, breathing or gastrointestinal effects during the observation period. Although 3 deaths were recorded at some points in this study, they were considered possibly related to the administration of the study drug to the wrong tube of the animals. The LD_{50} of the different constituents of the polyherbal formulation is therefore greater than 3200 mg kg^{-1} orally.

Effects of polyherbal co-treatment on coagulation dyscrasias: Compared to the control group (0.90), the PT-INR of 2-BE treated animals was significantly ($p<0.001$) increased (1.50). Co-treatment of polyherbal with 2-BE attenuated this increase in the PT-INR in group C, D and E while there was a non-significant ($p>0.005$) change in the PT-INR of rabbits treated with the polyherbal formula alone (Fig. 1a).

The treatment with 2-BE (Group B) caused a highly significant ($p<0.001$) increase in the value of aPTT (105.67) when compared to the control ones (41.33). However, there was a significant ($p<0.001$) decrease in the aPTT of the polyherbal-treated rabbits C, D and E compared to the 2-BE treated rabbits (Group B). A slight decrease in the aPTT of the polyherbal formula alone group (Group F) was also seen compared to the control (Fig. 1b).

Polyherbal administration reversed elevated Proinflammatory biomarker: There was a highly significant

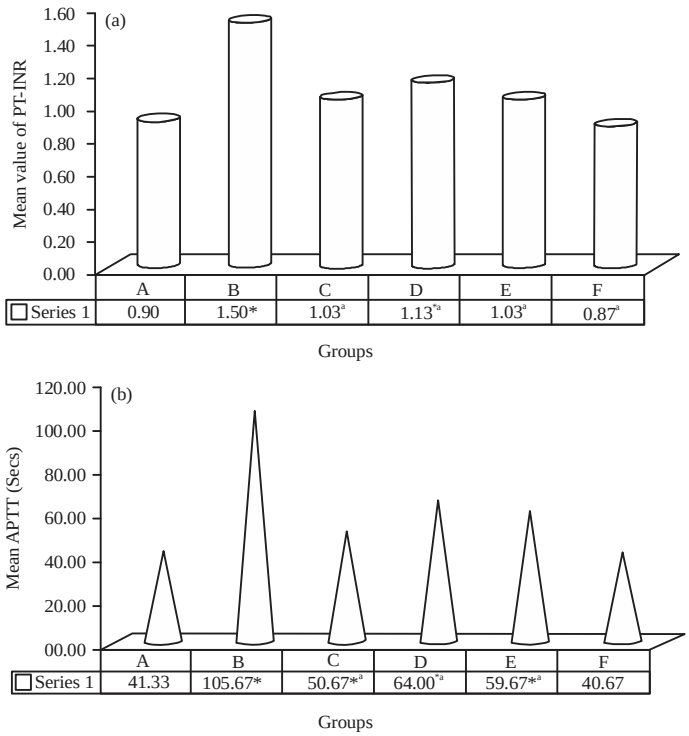

Fig. 1(a-b): Effects of polyherbal formulation treatment on the markers of haemostatic dysfunction in rabbits, (a) Levels of PT-INR, and (b) Levels of aPTT

Table 2: Results of the phytochemical evaluation of extracts of *H. umbellata*, *C. portoricensis*, *K. africana*, *L. breviflora* and *N. latifolia*

Tests	*H. umbellate*	*C. portoricensis*	*K. africana*	*L. breviflora*	*N. latifolia*
Phenol	-	-	++	+	-
Tannin	+	-	++	+	+
Steroid	-	+	-	-	-
Cardiac glycoside	+	+	-	-	-
Reducing sugar	++	-	+	-	-
Terpenoid	-	-	+	+	-
Alkaloid	++	-	++	+	+
Flavonoid	+	-	+	+	+
Saponin	++	+	++	+	+
Glycoside	-	+	++	-	+
Volatile oils	+	-	-	-	-
Fatty acids	-	+	-	-	-
Carotenoids	-	-	-	+	-
Oxalates	-	-	-	+	-
Phytate	-	-	-	+	-

+: Present in little concentration, ++: Present in very high concentration, -: Absent

($p<0.001$) increase in the mean value of circulating CRP in the plasma of 2-BE treated rabbits (62.23) compared to that of the control (9.16). Co-treatment with polyherbal resulted in a highly significant ($p<0.001$) decrease in the CRP of groups C, D and E, respectively compared to the 2-BE treated rabbits (Fig. 2).

The 2-BE treated rabbits showed positive correlation between PT-INR and CRP (R = 0.77). PT-INR and CRP have moderate negative association (-0.643) while aPTT and CRP have very strong and significant positive association (0.998*). The combined effects of all the treatments showed CRP and coagulation indices have strong positive relationship (Table 3). The overall effects show that there was a significant cumulative correlation of PT, aPTT and platelet indices in all the treatment groups in the study.

Polyherbal co-treatment effects on tissue histology: Histological examination of the liver and spleen sections

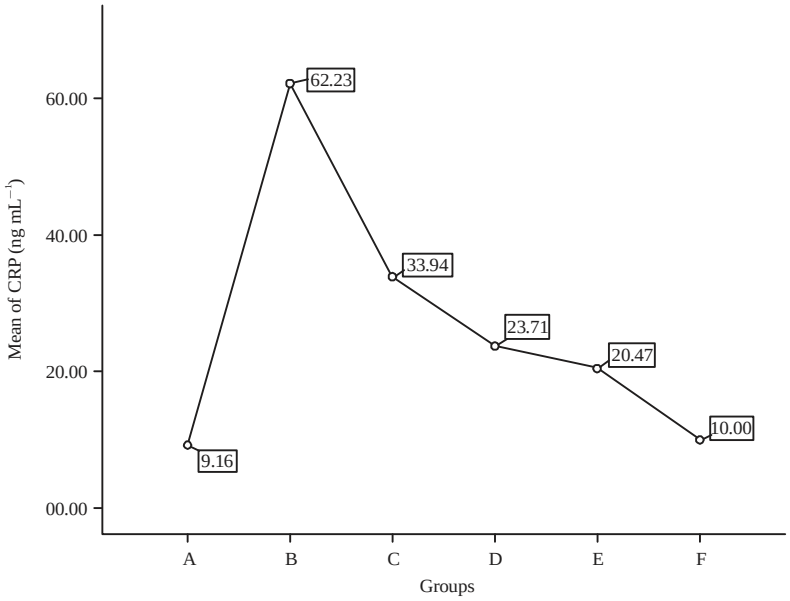

Fig. 2: Effects of the Polyherbal mixture on CRP as an inflammatory biomarker among the treated groups. All data are expressed as Mean±SEM (n = 3) and significantly different at $p<0.05$

Table 3: Correlation between the coagulation indices and CRP among the various treatment groups

	Coagulation indices	
Groups	PT-INR	aPTT (Secs)
CRP (ng mL^{-1})		
B	0.773	-0.977
C	-0.643	0.998*
D	0.868	0.690
E	0.952	-0.121
F	0.896	-0.064
ALL	0.927**	0.912**

Values are expressed as Mean±SEM (n = 3) and significantly different at p = 0.05

revealed the disruptive effects of 2-BE on the organs morphology. Compared with negative control, 2-BE-treated liver at 48 h after oral administration showed moderate necrosis with congested blood vessel (indicated by arrows) in the triad and disruption of cellular integrity. Such necrosis and organ disruption was not observed in the polyherbal-co-treated liver (Fig. 3a). 2-BE-treated spleen 48 h after oral administration showed marked congested blood vessel (indicated by arrows) in the splenic structure, due to thrombosis along the arterial supply (Fig. 3b), accompanied by splenic sequestration of possibly haemolysed red cells, this was not found with the polyherbal-co-treatment.

DISCUSSION

This Present study analyzed the effect of a polyherbal formulation on some basic biomarkers of coagulation by assessing circulating levels of coagulation factors, platelet indices and C-reactive protein in the plasma of control and experimental treatment groups. The suppression of the rapid consumption of the coagulation biomarkers in polyherbal treated groups suggests that the phytochemicals in this formula has the ability to enhance the clearance of 2-BE induced thrombosis, microvascular occlusions and reversed the haemolysis. 2-butoxyethanol (2-BE) has been reported to cause thrombus formation, lysis of the red cells and infarction having close similarities to the complications that have been observed in haemolytic disorders and haemoglobinopathy[25-27]. Although the mechanism of the haematotoxicity observed with 2-BE is not the same as the mechanism of the haemoglobinopathy disease states, the complications associated with thromboembolism and the coagulation profile observed in previous studies have a strong semblance to the pathological state which is observed in the human diseased states[7,26,28]. Thromboembolism and disseminated thrombosis involves the apprehension and rapid consumption of a number of certain coagulation factors[29,30] responsible for the initiation and formation of clots upon activation. It is, therefore, possible that deactivation of the coagulation factors by polyherbal formulation, might account for the decrease in the coagulation indices observed (Fig. 1a-b).

Assessment of thrombogenesis and thromboembolism is an apparently sensitive and quick procedure for evaluating the degree of thrombose formation, consumption of the clotting

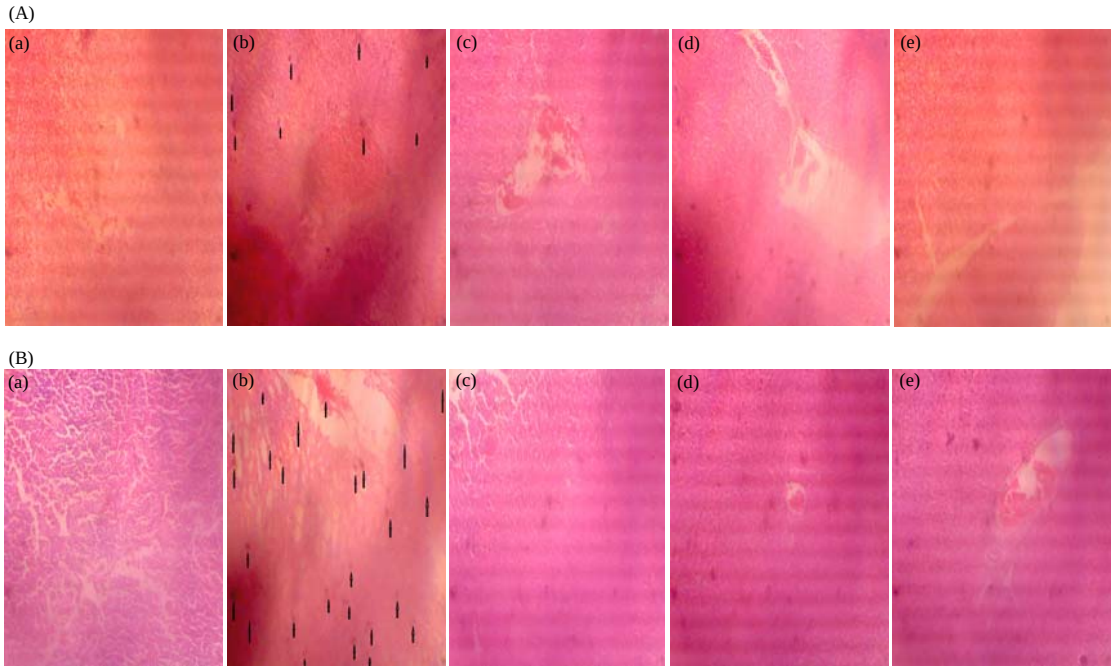

Fig. 3 (a-b): Histological examination of the rabbit liver and spleen after 2-BE induction or 2-BE and polyherbal cotreatment compared with naive/negative control

factors and for treatment monitoring. Coagulation factors are responsible for the initiation and formation of clots in cases of trauma to the blood vessels or skin, the deficiency of which can lead to clotting inefficiencies and hyperactivity or excess of which results in subsequent stroke as is the case with thromboembolism[31]. Lately, a number of studies have shown the wide use of medicinal plants in the treatment of haemoglobinopathies specifically Sickle Cell Disease and its complications[32,33], however knowledge of their anti-sickling, anti-thrombotic and anti-inflammatory activities are currently limited. The various herbs that constitute the polyherbal formula used in this study have been reported to possess different phytochemicals such as flavonoids, alkaloids and other secondary medicinal metabolites that have all been associated with anti-inflammatory, anti-thrombotic and antioxidant actions when given in combination[34]. The drastic increase in coagulation indices and platelet index dyscrasias of the 2-BE-treated (haemoglobinopathy) group, has been remarkably counteracted by the polyherbal formulation, thus justifying its significant role and usage for pro-thrombotic and haemoglobinopathy conditions in the traditional medicine settings[28,35].

Furthermore, data represented in Fig. 2 showed an altered serum CRP expression represented by a significant increase in the mean value of CRP as a pro-inflammatory biomarker in the 2-BE treated rabbits compared to the control group. Infection and inflammation have both been reported to cause excessive production of thrombopoietic factors that act on the precursors of megakaryocytes and the megakaryocytes[36]. CRP and IL-6 are pro-inflammatory thrombopoietic factors and plasma levels of which are elevated in cases of reactive thrombocytosis which may be key mediators of increased thrombopoietin and consequent reactive thrombocytosis[37]. The pro-inflammatory markers directly affect endothelial lining subsequently leading to the expression of tissue factor which initiates production of thrombin, thereby promoting coagulation. Tissue factor has been discovered to be a proinflammatory mediator itself[38-40]. In the course of this study, the groups treated with 2-BE (particularly groups B, D and E) were observed to have had elevated body and anal temperatures upon induction. The Polyherbal formulation reduced the activities of peripheral CRP in circulation, 2-BE-induced thrombosis, thrombophillia, normalized platelet indices while lowering the body and anal temperatures in the Polyherbal co-treatment groups (C, D, E and F) compared to the 2-BE treated rabbits (Fig. 1 and 2). Chakraborty and colleagues reported that plant flavonoids aim for the prostaglandins which are involved in the late phase of acute inflammation and pain perception and can thus be suggested that the presence of these flavonoids is likely influential in the anti-inflammatory and anti-thrombotic properties which the aqueous polyherbal formula demonstrated[41,42]. This may, therefore, explain why the expression of circulating CRP and coagulation indices were down-regulated, the polyherbal's ability as a vascular protective agent and as a major

mechanism through which it combats endothelial dysfunction associated with inflammation. This finding correlates with other studies on the effect of the polyherbal constituents on humans and animal models[13,42,43].

This study revealed that the combined effects of all the treatment groups showed CRP and coagulation indices have strong positive relationship (0.927** for PT and 0.912** for aPTT) as the 2-BE treated rabbits showed significant positive association between PT-INR and CRP (Table 3), also Fig. 1b showed a significant increase in the aPTT values of 2-BE treated rabbits compared to the negative control group. A major consequence of inflammation is endothelial dysfunction, tissue injury and subsequent haemostatic derangement which occurs in prolong inflammation accompanied with oxidative stress[44]. This correlation between CRP and coagulation indices can be explained by the activation of the tissue factor and the enhancement of the adherence of red blood cells to the endothelial cells of the endothelium in response to 2-BE, which could induce thrombophillia and disseminated thrombosis in the treated subjects[26,45]. 2-BE-induced haemoglobinopathy complications has been demonstrated in this study by the increase in the values of the evaluated coagulation assays, PT-INR and aPTT as these assays are a function of various plasma proteins regulated by series of orchestrated events which are dependent on the vessel walls as well as numerous proteins and cells[25,46,47]. Meanwhile, results from this present study confirmed a significant improvement in both the aPTT and CRP by decreasing the values of these parameters in the polyherbal co-treated rabbits (C, D and E) compared to the 2-BE treated rabbits (Group B). The restorative effect of the polyherbal on 2-BE suppressed coagulation indices might be due to direct effects on the endothelial cells by the antioxidant, anti-inflammatory and anti-thrombotic activities of the polyherbal constituents[34,48].

Histological examination of the animal tissue sections revealed a moderate necrosis with congested blood vessel of the 2-BE treated rabbit liver presumably due to the disruption of cellular integrity, ongoing haemolysis and thrombotic dissemination along the microvascular circulation of the animals[49]. Likewise, the spleen showed marked congested blood vessel (indicated by arrows) in the splenic structure, due to thrombosis along the arterial supply, accompanied by splenic sequestration of possibly haemolysed red cells due to the ongoing haemolysis compared to that of control (Fig. 3b). Previous studies reported that these same organs affected by thrombosis and haemolysis in the animal models (heart, lungs, liver, brain, liver, bones, eyes, spleen and liver) is comparable to what has been observed in the manifestations of the sickle cell disease and other haemoglobinopathies in human[25,50]. Also, 2-BE has been reported to result in disseminated thrombosis in various tissues, vessels and organs associated with microvascular complications in female rats after being exposed to it. Treatment with chemical agent, 2-BE resulted in atrophy of the scrotum of the male rabbits suggestive of disseminated thrombosis along the arterial supply to this region leading to occlusion of the micro-vessels supplying the organ and eventual tissue ischaemia similar to the complications of haemoglobinopathy[51,52]. These alterations were however, prevented and reverted to near normal by pretreatment and post-treatment with the polyherbal formulation. This finding correlates with other studies on the effect of the active phytochemical constituents of individual plants in polyherbal formulation on humans and animal models[15,53,54].

Major limitation was the cumbersome nature of the study as it took us a longer time in administering the 2-BE and the polyherbal to the rabbits in order to prevent asphyxiation by wrong passage of fluids. Further research to refine the extraction of procedure of the polyherbal could lead to the development of improved pharmaceutical products to manage and treat these common complications of haemoglobinopathies such as sickle cell disease.

CONCLUSION

The findings of this study revealed that the polyherbal formulation did not cause any metabolic toxicity, attenuated the level of hypercoagulability and circulating thrombose thereby mitigating the associated endothelial dysfunction in addition to its antioxidant and anti-inflammatory activities when administered to rabbits exposed to 2-BE-induced haemoglobinopathy complications. However, further studies are needed to elucidate the polyherbal molecular mechanisms involved in its therapeutic role as an anti-thrombotic fibrinolytic agent.

STATEMENT OF SIGNIFICANCE

Identified in this study were some mechanisms supporting the folkloric use of the polyherbal formula in most communities of Nigeria and parts of West Africa for the management of hypercoagulability, inflammation, cardiovascular problems and stroke, all complications associated with haemoglobinopathies. The polyherbal formula presented with anti-thrombotic, thrombolytic, anti-inflammatory and antioxidant activities when administered at 1 mL kg^{-1} of 100 mg mL^{-1} dosage. The

therapeutic effects of the polyherbal attenuated the level of hypercoagulability and circulating thrombose simultaneously, thus mitigating the associated endothelial dysfunction. This study provides an important basis for further investigation into the isolation, identification, characterization and molecular mechanisms behind the specific thrombolytic activity of the bioactive anti-thrombotic and anti-inflammatory phytochemicals present in the polyherbal formula and to emphasize the use of traditional medicine in the management and treatment of common complications of haemoglobinopathies by Scientists and Physicians.

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