Journal of Medicinal Plants Research Vol. 6(17), pp. 3388-3392, 9 May, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.449
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Comparative studies on anticoagulant properties of aqueous leaf extract of *Euphorbia heterophylla* and ethylene diamine tetra acetic acid (EDTA)

Ughachukwu, P. O.[1]*, Ezenyeaku C. C. T. [2], Ogamba J. O.[3], Onwurah W. O.[4] and Ifediata F. E.[4]

[1]Department of Pharmacology and Therapeutics, College of Medicine, Anambra State University; Awka Campus, Anambra State, Nigeria.
[2]Department of Obstetrics and Gynaecology, Anambra State University Teaching Hospital, Amaku Awka, Anambra State, Nigeria.
[3]Department of Pharmacology and Therapeutics, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.
[4]Department of Haematology and Blood Group Serology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria.

Accepted 28 March, 2012

Laboratory anticoagulants have certain drawbacks. For example, heparin has no preservative property on whole blood because its anticoagulant properties are neutralized by plasma while ethylenediaminetetraacetic acid (EDTA) is toxic and damages platelets. The search for novel anticoagulants therefore became necessary. The effect of the extract of *Euphorbia heterophylla* and tripotassium ethylene diamine tetracetic acid (K_3EDTA) on haemoglobin, platelets, red blood cells, white cell count and differentials were compared. 5 g of aqueous leaf extracts of *E. heterophylla* was prepared using Soxhlet extracting machine. Serial dilutions of the extract were made using distilled water. 0.5 ml of each dilution was mixed with 2 ml of whole human blood and stored for 5 days at 4℃. For control, this storage was repeated with EDTA and analyzed for HB, PLT, RBC, WBC, NEUT and lymph using Haematology autoanalyser (Erma Inc, PCE – 210). At $p < 0.05$ using students t-test, there was no statistically significant difference on HB, PLT, RBC, WBC, NEUT and lymph counts (p values = 0.13, 0.74, 0.058, 0.31, 0.086, 0.096, respectively). It was concluded that the aqueous leaf extract of *E. heterophylla* and K_3EDTA have comparable effect on HB, PLT, RBC, WBC, NEUT and lymph counts when used as storage anticoagulants.

Key words: Tripotassium ethylene diamine tetracetic acid (K_3EDTA), *Euphorbia heterophylla*, anticoagulants, storage, WBC, platelets, RBC.

INTRODUCTION

Anticoagulants are substances that are used to prevent formation of blood clots. Uses of anticoagulants include prevention of blood clots within the cardiovascular system, (heparin, warfarins), preservation of stored whole blood for transfusion (acid, citrate dextrose, citrate phosphate dextrose), preservation of blood samples for haematological tests [ethylenediaminetetraacetic (EDTA)].

*Corresponding author. E-mail: ughapieces@yahoo.com. Tel: 08061146275.

Laboratory anticoagulants have certain drawbacks. For example, heparin has no preservative property on whole blood because its anticoagulant properties are neutralized by plasma. It causes cells to dump and gives a blue background to blood films as described by Cheesbrough (2000). It is also too expensive. EDTA is not used for preserving blood for transfusion because it is toxic and damages platelets. Forscher et al. (1985) observed that some patients have antibodies that bind to platelets that are activated by EDTA, causing platelets clumping.

Progressive cellular changes occur in stored whole blood

over time. For instance, Lipp et al. (2005) demonstrated significant changes in haematologic parameters in blood samples stored for more than 24 h at 4℃. Specifica lly, Ahmed and Orakah (2009) found those leucocytes and platelets values fell from 6.7 × 10^9 and 253 × 10^9/L, respectively to critical values of 3.0 × 10^9 and 100 × 10^9/L after two days of storage at 4℃. These progressive cellular changes compromises the usefulness of blood stored for transfusion. The relative lack of blood fractionation technology to harvest specific blood components further compounds the problem of patients who receive blood and blood components. There is therefore need for novel anticoagulants with better haematological profile. Medicinal plants readily provide sources for such novel drugs discovery. Herbs such as *Zingiber officiale, Ginkgo biloba, Allium sativum, Panax ginseng* and *Synclisia scabrida* have anticoagulant properties as demonstrated by Afonne et al. (2000), Tattelman (2005) and Mousa (2010). Unekwe et al. (2006) had earlier demonstrated the anticoagulant properties of aqueous leaf extract of *Euphorbia heterophylla*.

The present study seeks to find the effect of this extract on blood cells when it is used as an anticoagulant. Specific objectives include:

(a) To determine the effect on HB, RBC, WBC and platelets counts after anticoagulation with this extract and storage at 4℃ for five days.
(b) To compare the effect on HB, RBC, WBC and platelets counts when this extract is used as storage anticoagulant with that of K_3EDTA.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *E. heterophylla* were collected in the month of June 2010 at a farm in Ifitedunu, Anambra State Nigeria. The plant was identified by Dr. D. Mbaezue of Botany Department, Nnamdi Azikiwe University Awka, Nigeria and a specimen deposited at the university herbarium for future references.

Preparation of aqueous extract

Fresh leaves of *E. heterphylla* were washed with clean water and air dried at room temperature for ten days. Thereafter, 200 g of the dry leaves was mashed in 250 ml distilled water and left for 24 h. Thereafter, it was filtered and the filtrate evaporated to dryness using Soxhlet extracting machine. Phytochemical test was performemed on the aqueous extract.

Preparation of solutions of extract

4 g of extract was dissolved in 10 ml of distilled water giving a stock solution of 0.4 g/ml (400 mg/ml). From the stock, serial dilutions 1:1 (200 mg/ml), 1:2 (130 mg/ml), 1:3 (100 mg/ml), 1:4 (80 mg/ml) and 1:5 (65 mg/ml) were made using distilled water.

Collection of blood samples

Blood samples were collected from twenty subjects who were randomly selected from patients coming for routine hematological investigations at Haematology and Serology Unit of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria after their consents were obtained.

Anticoagulation experiment

0.5 ml of 200 mg/ml dilution was placed in 3 specimen bottles and labeled E_1 (in triplicates). This was repeated for 130, 100, 80 and 65 mg/ml dilutions and labeled E_2, E_3, E_4, E_5, respectively. 2 ml of fresh human whole blood from different subjects were added to each of the extract-containing specimen bottles and gently shaken for proper mixing. For control, 2 ml of fresh human whole blood from five different subjects were added to five standards EDTA bottles (K_3EDTA) at concentrations of 1.5 mg EDTA/ml whole blood and labeled C_1, C_2, C_3, C_4, C_5. The samples were also shaken gently for proper mixing. All samples and control were stored at 4℃ for five days. Thereafter, the samples and control were analyzed for haematocrit (PCV), mean cell volume (MCV), mean cellular haemoglobin (MCH), mean cellular haemoglobin concentration (MCHC) using Haematology autoanalyser (Erma Inc, PCE – 210).

Statistical analysis

Figures obtained for the sub-groups were used to calculate the mean values for each group. The mean values for the K_3EDTA-preserved group (group C) were compared with those of the extract preserved group (group E) and the figures regarded as significant at $p < 0.05$ using students t-test.

RESULTS

Final weight of extract was 5 g, giving percentage yield of 2.5. Phytochemical test on the aqueous extract showed the presence of saponins, tannins, starch, alkaloids, flavonoids and reducing sugars. This extract had preservative effect on HB, PLT, RBC, WBC, NEUT and lymph counts when used as an anticoagulant for storing whole blood at 4℃ for five days as shown in Table 1. It also had preservative effect on blood cells and platelets comparable to that of standard anticoagulant (K_3EDTA) as shown in Tables 2 and 3. At $p < 0.05$, there was no statistically significant difference on HB, PLT, RBC, WBC, NEUT and lymph counts (p values = 0.13, 0.74, 0.058, 0.31, 0.086 and 0.096, respectively) when K_3EDTA and extract-figures were subjected to students t-test analysis. Blood samples were anticoagulated with aqueous leaf extract of *E. heterophylla* and stored for five days at 4℃. Control blood samples were anticoagulated with K_3EDTA and equally stored for five days at 4℃. Thereafter, the samples and control were analysed for HB, PLT, RBC and WBC counts using Haematology autoanalyser (Erma Inc).

DISCUSSION

At $p < 0.05$, there was no statistically significant difference

Table 1. Mean values (±S.E.M.) of Hb, PLT and WBC counts in human whole blood after anticoagulation with aqueous leaf extract of *E. heterophylla* (n = 3).

Parameter	E1	E2	E3	E4	E5	Normal values
HB (g/dl)	6.6 ± 2.44	9.6 ± 3.00	8.7 ± 2.83	9.5 ± 3. 00	11.8 ± 3.46	12.5 – 15.5
PLT (× 10^3/µl)	128 ± 11.62	130 ± 11.22	284 ± 16.82	233 ± 16.2 2	122 ± 10.86	150 – 400
RBC (× 10^6/µl)	2.6 ± 1.41	3.7 ± 1.73	2.4 ± 1.41	3.4 ± 1.73	4. 4 ± 2.00	3.8 – 6.5
WBC (×10^3/µl)	6.9 ± 1.41	7.4 ± 2.64	5.6 ± 2.24	5.8 ± 2.23	9. 0 ± 3.16	4.0 – 11.0
NEUT (%)	43.0 ± 6.56	38.3 ± 6.63	25 ± 4.90	25 ± 5.6 5	34.7± 6.14	40 - 75
LYMP (%)	57 ± 7.55	61.7 ± 7.45	75 ± 8.72	75 ± 8.23	65.3 ± 7.87	20 - 45
MONO (%)	–	–	–	–	–	2 - 10
EOSINO (%)	–	–	–	–	–	1 - 6
BASO (%)	–	–	–	–	–	< 1

Table 2. Values of HB, PLT, RBC and WBC counts in human whole blood after storage with aqueous leaf extract of *E. heterophylla* and K_3EDTA, respectively.

Parameter	EDTA (control)					EXTRACT (samples)				
	C1	C2	C3	C4	C5	E1	E2	E3	E4	E5
HB (g/dl)	8.6	12.6	10.3	11.5	12.9	6.6	9.6	8.7	9.5	11.8
PLT (x 10^3/µl)	299	126	159	261	134	128	130	284	233	122
RBC (x 10^6/µl)	3.8	5.2	3.3	4.9	5.4	2.6	3.7	2.4	3.4	4.4
WBC (x 10^3/µl)	5.9	6.0	7.4	5.1	6.4	6.9	7.4	5.6	5.8	9.0
NEUT (%)	58	58	39	29	46	43	38	25	25	35
LYMP (%)	42	42	61	71	54	57	61	75	75	65
MONO (%)	–	–	–	–	–	–	–	–	–	–
EOSINO (%)	–	–	–	–	–	–	–	–	–	–
BASO (%)	–	–	–	–	–	–	–	–	–	–

Blood samples were anticoagulated with aqueous leaf extract of *E. heterophylla* and stored for five days at 4℃. Control blood sam ples were anticoagulated with K_3EDTA and equally stored for five days at 4℃. There after, the samples and control were analysed for HB, PLT, RBC and WBC counts using haematology autoanalyser (Erma Inc, PCE – 210).

Table 3. Mean values (±S.E.M.) of HB, PLT, RBC and WBC counts in human whole blood after storage with aqueous leaf extract of *E. heterophylla* and K_3EDTA, respectively (n = 5).

Parameter	K_3EDTA (control)	Extract (samples)	Normal values
HB(g/dl)	11.2 ± 1.6	9.2 ± 1.3	12.5 – 15.5
PLT(x10^3/µL)	195.8 ± 32.2	179 ± 25.4	150 – 400
RBC(x10^6/µL)	4.5 ± 0.9	3.3 ± 0.7	3.8 – 6.5
WBC(x10^3/µL)	6.1 ± 1.1	6.9 ± 1.3	4.0 – 11.O
NEUT(%)	39.6 ± 5.8	33.2 ± 4.1	40 – 75
LYMPH(%)	54 ± 5.9	66.8 ± 4.8	20 – 45
MONO(%)	–	–	2 – 10
EOSIN(%)	–	–	1 – 6
BASO(%)	–	–	< 1

Blood samples were anticoagulated with aqueous leaf extract of *E. heterophylla* and stored for five days at 4℃. Control blood sam ples were anticoagulated with K_3EDTA and equally stored for five days at 4℃. There after, the samples and control were analysed for HB, PLT, RBC and WBC counts using haematology autoanalyser (Erma Inc, PCE – 210).

values 0.13, 0.74, 0.058, 0.31, 0.086 and 0.096, respectively). The minimum concentration of the extract required for anticoagulation was 40 mg/ml whole blood compared to 1.5 mg/ml for K_3EDTA. The apparently

higher efficacy of K_3EDTA could be because this extract, being unrefined could contain antagonizing bioactive principles as previously observed by Kafaru et al. (1994). The extract, like K_3EDTA, reduced monocytes, basphils and eosinophils counts but preserved HB, RBC, and PLT as shown in Tables 1 to 3. This agreed with earlier findings by Baca et al. (2006). Herdberg and Lehto (2009) had shown that haematological parameters in anticoagulated blood were usually stable at room temperature for only about 48 to 72 h. Zandecki et al. (2007) had earlier shown that spuriously low WBC counts may be observed because of agglutination in the presence of EDTA, cryoglobulins and lipids in stored whole blood. Also, Kim et al. (2008) demonstrated that blood samples containing yeast, bacteria and other contaminants may give erroneously low WBC and differential counts by causing the cells to clump together especially when the analysis is done by automated haematology autoanalysers.

Both the extract and K_3EDTA preserved blood samples showed increased lymphocyte counts (Tables 1 and 3). The relative lymphocytosis could be explained by reduced monocyte, eosinophil and basophil counts as lymphocytes are usually stable on storage with EDTA for five days as observed by Lloyd (1982). Reduced monocyte count could also be due to necrosis of these nucleated cells after storage as demonstrated by Lam et al. (2004). The HB, RBC and total WBC counts were preserved. This agreed with earlier findings by Reardon et al. (1991). Both the extract and K_3EDTA did not increase or decrease platelet count when used as storage anticoagulant (Table 3). This contrasted earlier findings by Olsen et al. (2001) who found significant increase in platelet count when anticoagulated blood is stored at 5℃, but normal values when it is stored at room temperature for 24 h. Faraday and Rosenfeld (1998) felt that this could probably be because reduced temperature induced platelet activation and fibrinogen binding. However, this assumption that platelets are more stable at room temperature was not supported by Maurer-Spurej et al. (2001) who demonstrated that room temperature significantly activated platelets by increasing activation of platelet activation marker GPIIb-IIIa as measured by flow cytometry. The ability of this extract to maintain normal platelet values when used as storage anticoagulant means that, it can be used to store blood samples for serum potassium estimation. Pseudothrombocytosis leads to spuriously high potassium concentration since thrombocytosis is one of the several pre-analytical factors for serum potassium concentrations (Thurlow et al., 2005).

Conclusion

It could be concluded that the aqueous leaf extract of *E. heterophylla* had preservative effect on HB, PLT, RBC, WBC and NEUT counts when used as an anticoagulant to store human whole blood for five days. It could also be concluded that both extract and EDTA had comparable preservative effect on these parameters. This extract should therefore be explored as an alternative laboratory anticoagulant. Further studies are needed to determine the effect of anticoagulation with this extract on PCV, MCV, MCH and MCHC. Also acute and chronic toxicity studies on this extract are needed to determine its safety as anticoagulant for preserving blood for transfusions.

REFERENCES

Afonne OJ, Orisakwe OE, Obi E, Orish C, Akumba DD (2000). Some Pharmacological properties of *Synclisia scabrida* III. Indian J. Pharmacol., 32: 239-241.

Ahmed SG, Orakah JA (2009). Cellular changes in stored whole blood and the implication on efficacy of transfusion therapy in Nigeria. The Internet Journal of Third World Medicine 8(2). Available at: http://www.ispub.com/journal/the-internet-journal-of-third-world-medicine/volume-8-number-2/cellular-changes-in-stored-whole-blood-and-the-implication-on-efficacy-of-transfusion-therapy-in-nigeria.html . (Accessed March 25, 2012)

Baca ME, Gulati G, Kocher W, Schwarting R (2006). Effect of storage of blood at room temperature on hematologic parameters measured on Sysmex xe-2100. Lab. Med., 37(1): 28-36.

Cheesbrough M (2000). District laboratory practice in tropical countries. (part 2) Cambridge. Cambridge University Press, pp. 297-298.

Faraday N, Rosenfeld BA (1998). In vitro hypothermia enhances platelet GPIIb-IIIa activation and p-selectin expression. Anaesthesiol., 88(6): 1579-1585.

Forscher CA, Sussman II, Friedman EW (1985). Pseudothrombocytopenia masking true thrombocytopenia. Am. J. Hematol., 18: 313-17.

Herdberg P, Lehto T (2009). Aging stability of complete blood count and white blood cell differential parameters analyzed by Abbott-DYN Sapphire haematology analyzer. Int. J. Lab. Haematol., 31(1): 87-96.

Kafaru CJ, Keay MB, Roper ZF (1994). Flavonoids: antibacterial constituents of *Vitex doniana*. J. Nat. Sci., 61: 80-81.

Kim HR, Park BR, Lee MK (2008). Effects of bacteria and yeasts on WBC counting in three automated haematology counters. Ann. Hematol., 87(7): 557-62.

Lam NYL, Rainer TH, Chiu RWK, Denis LYM (2004). EDTA is a better anticoagulant than heparin or citrate for delayed blood processing for plasma DNA analysis. Clin. Chem., 50: 256-257.

Lipp G, Salvaqno GL, Solero GP, Franchini M, Guidi GC (2005). Stability of blood cell counts haematolocal parameters and reticulocyte indexes on the Advia A120 haematologic analyzer. J. Clin. Med., 146(6): 333-340.

Lloyd E (1982). The deterioration of leucocytes morphology with time: Its effect on the differential count. Lab. Perspect., 1(1): 13-16.

Maurer-Spurej E, Pfeiler G, Maurer N, Lindner H, Glatter O, Devine DV (2001). Room temperature activates human blood platelets. Lab. Invest., 81: 581-92.

Mousa SA (2010). Antithrombic effects of naturally derived products on coagulation and platelet function. Methods Mol. Biol., 663: 229-40.

Olsen AK, Bladbjerg EM, Jensen AL, Hansen AK (2001). Effect of pre-analytical handling on hematological variables in mini pigs. Lab. Anim., 35(2): 147-52.

Reardon DM, Warner B, Trowbridge EA (1991). EDTA, the traditional anticoagulant of haematology: with increased automation, is it time for a review? Med. Lab. Sci., 48: 72-75.

Tattelman E (2005). Health effects of garlic. Am. Fam. Physician, 72 (01): 103-106.

Thurlow V, Ozevlat H, Jones SA, Bailey IR (2005). Establishing a practical blood platelet threshold to avoid reporting spurious potassium results due to thrombocytosis. Ann. Clin. Chem., 42(3): 196-199.

Unekwe PC, Ughachukwu PO, Ogamba JO (2006). Some

pharmacological studies of aqueous extract of leaves of *Euphorbia heterophylla*. Trop. J. Med. Res., 10 (2): 1-5.
Zandecki M, Genevieve F, Gerard J, Godon A (2007). Spurious counts and spurious results on haematology analyzers: a review. Int. J. Lab. Hematol., 29(1): 21-41.