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Effect on some haematological indices of human whole blood when aqueous leaf extract of *Euphorbia heterophylla* was used as storage anticoagulant

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Properties of red blood cells, especially cell size and red cell indices related to size, change with time in stored blood samples. Laboratory anticoagulants have certain drawbacks. For example, heparin has no preservative property on whole blood while K_3EDTA (EDTA) is toxic and damages platelets. The search for novel anticoagulants with better hematological profile is therefore necessary. The anticoagulant properties of aqueous leaf extract of *Euphorbia heterophylla* (aka ito in Igbo) were compared with those of K_3EDTA. Specifically, the effect of this extract and K_3EDTA on packed cell volume (PCV) and red cell indices related to size, when they were used as anticoagulants, were compared. 0.5 ml of serial dilutions of this extract were placed in specimen bottles containing 2 ml fresh human whole blood and stored for 15 days at 4°C. For control, 2 ml fresh human whole blood was added to specimen bottles containing 1.5 mg/ml EDTA and stored for 15 days at 4°C. Thereafter, the test and control samples were analyzed for PCV, MCV, MCH and MCHC using haematology autoanalyser (Erma Inc, PCE - 210). All concentrations of the extract used, except 65 mg/ml, and the K_3EDTA were able to keep the blood samples in fluid state throughout the 15 days period of storage. At the level of significance, $p < 0.05$, this extract had comparable preservative effect on MCV and MCH ($p = 0.79$; 0.20), but less preservative effect on PCV and MCHC when compared with EDTA ($p = 0.013$; 0.049). The aqueous leaf extract of *Euphorbia heterophylla* has preservative properties on haematological indices of stored human whole blood compare to that of K_3EDTA. The fact that it does not chelate calcium as its mechanism of anticoagulation gives it an advantage over K_3EDTA when used for biochemical tests. It should therefore, be explored as alternative laboratory anticoagulant in view of this advantage.

Key words: *Euphorbia heterophylla*, anticoagulants, storage of blood, red cell indices, K_3EDTA.

INTRODUCTION

Anticoagulants are substances that are used to prevent formation of blood clots. Their uses include prevention of blood clots (thrombosis) within the cardiovascular system (heparin and warfarins), preservation of stored whole blood

and blood fractions (acid citrate dextrose, citrate phosphate dextrose adenine) and preservation of blood samples for haematological analysis (EDTA, heparin).

Laboratory anticoagulants presently available have certain drawbacks. For example, heparin has no preservative property on whole blood because its anticoagulant properties are neutralized by leucoferrin and protamine sulfate in plasma (Mikalsi et al., 1978; Wu et al., 1995). It causes cells to clump and gives a bluish background to blood films (Cheesbrough, 2000; White and Escolar, 2000). EDTA is not used for preserving blood for transfusion because it is toxic and damages platelets. Use of blood and blood products is common in general medical practice as haemorrhage is known to be a leading cause of death especially in obstetrics (Irita and Inada, 2011). Studies on improvement of blood transfusion and its facilities are therefore of interest to all healthcare practitioners. PCV is used to calculate mean cell heamoglobin concentration (MCHC), mean cell haemoglobin (MCH) and mean cell volume (MCV); parameters used in assessment of anaemia. These haematological indices are affected by the type of anticoagulant used, the length and temperature of storage (Philips et al., 1998). There is therefore need for novel anticoagulants with better haematological profile.

Medicinal plants readily provide sources of such novel agents. Herbs known to have anticoagulant properties include *Zingiber officiale, Ginkgo biloba, Allium sativum, Panax ginseng* and *Synclisia scabrida* (Afonne et al., 2000; Tattelman, 2005; Mousa, 2010). Unekwe et al. (2006) had earlier demonstrated the anticoagulant action of the aqueous leaf extract of *Euphorbia heterophylla* (aka ito in Igbo). However, their study did not include the effect of this extract on red cell indices. The present study seeks to find the effect of the aqueous leaf extract of this plant on coagulation of stored human whole blood. Specific objectives include to study the effect of this extract on PCV, MCV, MCH and MCHC values of stored human whole blood, as well as to compare the effect of this extract and K_3EDTA on the above haematological indices on stored whole blood.

MATERIALS AND METHODS

Fresh leaves of *E. heterophylla* were collected in the month of June 2010 from Ifitedunu, Anambra State. The plant was identified by Prof. C.U. Okeke of Botany Department, Nnamdi Azikiwe University Awka, Anambra State, Nigeria. A specimen of this plant was deposited at the herbarium of this university for future references.

Preparation of aqueous extract

Fresh leaves of *E.heterophylla* were washed with clean water and air dried at room temperature for ten days. 200 g of the dry leaves was mashed in 250 ml of distilled water and left for 24 h.

Thereafter, it was filtered and the filtrate evaporated to dryness Using Soxhlet extracting machine. Percentage yield of extract was determined using the formula:

$$\% \text{ yield} = \frac{\text{Final weight of extract}}{\text{Weight of dry leaves}} \times 100$$

Phytochemical analysis

Phytochemical test was done on the extract to determine the bioactive principles as described by Harbone (1973).

Preparation of solution of extract

4 g of the extract was dissolved in 10 ml of distilled water giving a stock solution of 0.4 g/ml (400 mg/ml). From this stock solution 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.

Subject selection

Fifteen apparently healthy male blood donors aged between 25 to 30 years at Nnamdi Azikiwe University Teaching Hospital, Nnewi were recruited for this study after their informed consents were obtained. An ethical clearance was obtained prior to this study from the Medical Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi. All procedures were carried out in strict compliance with the Helsinki declaration.

Anticoagulation experiment

15 specimen bottles were divided into 5 groups of 3 bottles per group and labeled $E1_a$, $E1_b$, $E1_c$; $E2_a$, $E2_b$, $E2_c$; $E3_a$, $E3_b$, $E3_c$; $E4_a$, $E4_b$, $E4_c$; $E5_a$, $E5_b$ and $E5_c$, respectively. Thereafter, 0.5ml of serial dilutions 200, 130, 100, 80 and 65 mg/ml were placed in specimen bottles E1, E2, E3, E4 and E5, respectively. For the control, 15 standard EDTA bottles containing 1.5 mg K_3EDTA were divided into 5 groups of 3 bottles per group and labeled $C1_a$, $C1_b$, $C1_c$; $C2_a$, $C2_b$, $C2_c$; $C3_a$, $C3_b$, $C3_c$; $C4_a$, $C4_b$, $C4_c$; $C5_a$, $C5_b$ and $C5_c$. Then, 4 ml of whole blood was taken from the cubital vein of the forearm of the first donor using 5 ml syringe fitted with 21G needle after proper cleaning with spirit swab. 2 ml of the collected blood was immediately placed in $E1_a$ and the bottle gently shaken for proper mixing; the remaining 2 ml of whole blood was placed in $C1_a$ and gently shaken for proper mixing. This was repeated for the remaining fourteen donors corresponding to $E1_b$, $C1_b$; $E1_c$, $C1_c$; …….. $E5_c$, $C5_c$. All specimen bottles containing test and control blood samples were stored at 4°C for 15 days. Thereafter, the samples and control were analyzed for PCV, MCV, MCH and MCHC using Haematology autoanalyser (Erma Inc, PCE - 210).

Statistical analysis

Figures obtained for the groups (n=5) 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.

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Table 1. Mean values of PCV, MCV, MCH MCHC for group C (n=5) after anticoagulation of human whole blood with K_3EDTA and storage for 15 days at 4°C.

Parameter	C1	C2	C3	C4	C5	Mean	Normal range
PCV (%)	30.0	39.0	35.5	36.0	41.0	36.3 ± 1.87	(36-37)
MCV (fl)	79.0	75.0	106.0	78.0	76.0	82.8 ± 5.84	(82-85)
MCH (pg)	23.0	24.0	31.0	23.0	24.0	25.0 ± 1.52	(27-32)
MCHC (g/dl)	28.0	32.0	29.0	31.0	31.0	30-0 ± 0.62	(32-36)

Table 2. Mean values of PCV, MCV, MCH MCHC for group E (n=5) after anticoagulation of human whole blood with aqueous leaf extract of *E. heterophyla* and storage for 15 days at 4°C.

Parameter	E_1	E_2	E_3	E_4	E_5	Mean	Reference value
PCV (%)	22.0	28.3	26.7	26.3	34.3	27.5 ± 1.30	(36-47)
MCV (fl)	83.5	76.6	110.7	77.3	78.3	85.1 ± 6.47	(82-85)
MCH (pg)	25.3	26.0	36.3	28.0	27.0	28.5 ± 1.99	(27-32)
MCHC (g/dl)	30.0	33.3	32.0	35.6	34.0	32.9 ± 0.09	(32-36)

Table 3. Mean values(n=5) of PCV, MCV, MCH, MCHC obtained after anticoagulation of human whole blood with aqueous leaf extract of *E. heterophylla* (group E) compared with values obtained after anticoagulation with K_3EDTA (group C).

Parameter	Extract (Group E)	K_3EDTA (Group C)
PCV (%)	27.5 ±1.30	36.3 ± 1.87
MCV (fl)	85.1 ± 6.47	82.8 ± 5.84
MCH (pg	28.5 ± 1.90	25.0± 1.52
MCHC (g/dl)	32.9 ± 0.90	30.6± 0.62

RESULTS

The final weight of plant extract was 5 g; therefore percentage yield of extract from the dried leaves was 2.5%. Phytochemical analysis of the crude extract yielded the following constituents: saponins, tannins, reducing sugars, flavonoids, resins, alkaloids and cardiac glycosides. All concentrations of the extract used, except 65 mg/ml, were able to keep the blood samples in fluid state throughout the fifteen days period of study.

When values of MCV and MCH obtained from the test and control groups were subjected to t-test analysis, p values of 0.79 and 0.20 were respectively obtained. However, when values of PCV and MCHC were subjected to the same test, p values of 0.013 and 0.049 were respectively obtained. Table 1 shows mean values of PCV, MCV, MCH and MCHC for groups C_1-C_5 (n=5) after anticoagulation of human whole blood with K_3EDTA and storage for 15 days at 4°C. Table 2 shows mean values of PCV, MCV, MCH and MCHC for groups E_1-E_5 (n=5) after anticoagulation of human whole blood with aqueous leaf extract of *E. heterophylla*. Table 3 compares mean values(n=5) of PCV, MCV, MCH, MCHC obtained after anticoagulation of human whole blood with aqueous leaf extract of *E. heterophylla* with values obtained after anticoagulation with K_3EDTA and storage.

DISCUSSION

This study has shown that the aqueous leaf extract of *Euphorbia heterophylla* has significant anticoagulant properties. The minimum concentration of extract required for anticoagulation in this experiment was 80 mg / 2 ml whole blood compared to 1.5 mg / 2 ml whole blood for K_3EDTA. At concentrations used in this study, this extract had similar preservative effect on MCV and MCH when comparable with K_3EDTA ($p = 0.79$ and 0.20, respectively), but has less preservative effect on PCV and MCHC ($p = 0.013$ and 0.049, respectively), level of significance $p < 0.05$. It could be observed that the minimum concentration of the extract (80 mg / 2 ml) that exhibited anticoagulant effect on whole blood was much higher than the standard concentration of K_3EDTA (1.5 mg / 2 ml) required for anticoagulation. This apparent lower potency could be explained by the fact that this extract, being unrefined, contains many bioactive principles that could oppose the biochemical actions of one another (Inui et al., 2005).

The extract did not significantly affect the values of PCV, MCV, MCH and MCHC after storage at 4°C for fifteen days. This is in contrast to findings by Lawrence et al. (1975), Philips et al. (1998) and Hill et al. (2009) who recorded increased MCV, PCV and MCH after preservation with EDTA for 24 days. With regards to MCV, it must be observed that increased values after anticoagulation and storage for 24 days meant increase

in the size of red blood cells as a result of deterioration and swelling of the cells which increases with length of storage. Therefore, differences in length of storage could explain this difference in findings. It is also known that different haematology autoanalysers give varying haematological values (Gutierrez et al., 1996; Bourner et al., 2005; Imeri et al., 2008). Values of PCV and MCH follow the same pattern as that of MCV as they are derived values from MCV.

Temperature variations affect the value of haematological parameters in stored blood samples. Values for MCV slowly increase over time with either EDTA or heparin as anticoagulants when stored at room temperature as a result of swelling of red blood cells. This does not occur when the same sample is stored at 4°C for 24 days (Freise et al., 2009). Also, higher temperature causes increased PCV values when blood samples were stored at 24 and 33°C thus; reaffirming the fact that 4°C is the optimum temperature for storage of whole blood irrespective of the anticoagulant used (Hadzimusic et al., 2010).

As an anticoagulant, EDTA chelates calcium ions as its mechanism of action (Pal and Pal, 2005). It equally binds other ions in the blood. EDTA affects certain biochemical tests where calcium and other ions are involved. Imafuku et al. (2002) observed that when EDTA was used as an anticoagulant low values for serum alkaline phosphatase, calcium, and potassium ions were obtained. Chen et al. (2008) also demonstrated that in the determination of pharmacokinetic variables of tigecycline, the unbound fraction of the drug was higher in EDTA-treated blood because of competitive binding of ions by EDTA. This falsely affected the calculated clearance value of tigecycline. This extract contains saponin which inhibits blood coagulation by decreasing plasma fibrinogen (Oleszek and Marston, 2000). In this regard; this extract might have an advantage over EDTA as an anticoagulant when the blood sample is to be used for biochemical tests.

Conclusion

It has been demonstrated that the aqueous leaf extract of *E. heterophylla* has good anticoagulant and preservative effect on human whole blood as measured by some haematological indices. This anticoagulant effect compared favorably with that produced by K_3EDTA. This extract should, therefore, be explored as an alternative laboratory anticoagulant in view of its relative abundance in this environment. Further studies are needed to determine the effect of anticoagulation with this extract on total and differential white blood cell counts.

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