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OSMOTIC FRAGILITY INDEX OF HBAA RED BLOOD CELLS IN THE PRESENCE OF AQUEOUS EXTRACTS OF THREE MEDICINAL PLANTS *(AFRAMOMUM MELEGUETA, GARCINA KOLA,* AND *CYMBOPOGON CITRACUS).*

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ABSTRACT

In vitro study was carried out to ascertain the capacity of normal human adult red blood cells (HbAA genotype) to withstand hypotonicity in the presence of increasing concentrations of aqueous extract of three medicinal plants, *Aframomum melegueta, Garcina kola* and *Cymbopogon citracus.* Separate aqueous extracts of the three plant specimen in an increasing order of 0.2%, 0.4%, 0.6% and 0.8% (w/v) were used in this study. The osmotic fragility index of the red blood cells was determined with the use of spectrophotometric method and the result of the control and test samples were statistically compared (p value ≤0.05). The value of mean corpuscular fragility (MCF) (x ± S.D) of the control sample was 4.70 ± 1.7g/l while MCF value (x ± S.D) and the corresponding percentage stability of the red blood cells in the presence of 0.2%, 0.4%, 0.6% and 0.8% (w/v) of aqueons extracts of the three plant specimen were as follows: *A. melegueta* – 5.85 ± 1.1g/l (24.47%), 5.60 ± 1.3g/l (19.14%), 5.10 ± 0.9g/l (8.51%) and 5.20 ± 0.8g/l (10.64%): *G. kola* - 5.60 ± 2.2g/l (19.14%), 5.75 ± 2.1g/l (22.34%), 5.30 ± 1.2g/l (12.76%) and 5.70 ± 1.4g/l (21.28%) and *C. citracus* – 5.10 ± 2. 9g/l (8.51%), 4.90 ± 2.5g/l (4.26%), 5.00±2.1g/l (6.38%) and 5.10±2.2g/l (8.51%) . There were significant increases (p≤0.05) in the MCF values of the red blood cells in the presence of aqueous extract of the three medicinal plants. Therefore the results showed the capacity of aqueous extracts of *A. melegueta, G. kola* and *C. citracus* to enhance stability of red blood cell membrane which was indicated by reduced susceptibility of the red blood cells to lysis in the presence of these three plant extracts in hypotonic condition.

KEYWORDS: Osmotic fragility, *Aframomum melegueta, Garcina kola, Cymbopogon citracus,* means corpuscular fragility (MCF).

INTRODUCTION

A medicinal plant contains one or more substances that can be useful therapeutically or precursor molecules for drug synthesis (WHO, 1978). The biological and pharmacological properties often exhibited by extracts of these plants allow for traditional exploitation of this source for medicinal preparations. Clinically observed therapeutic benefits derived from such plants are usually contributed by their wide varieties of phyotochemicals, vitamin and mineral content (Okwu and Ekeke, 2003).

Aframomum melegueta (Alligator pepper) is a herbaceous perennial plant native to swampy habitant along the West African Coast. It's trumpet-shaped, purple flower develop into 5 – 7cm long pods containing numerous small reddish brown seeds. The seeds have a pungent, peppery taste due to presence of aromatic ketones e.g 1 – (4 hydroxy – 3 – methoxyphenyl) – decan – 3 – one (Okwu, 2005). Alcoholic extract of the seeds is used medicinally in the treatment of intermittent fever, dysentery and gastrointestinal trouble (Agoha, 1974).

Garcina kola (Bitter kola) seeds are served in Nigeria and other West African countries to guest as supplement to true kolanuts. The spherical fruit is about 7 – 12cm in diameter with dark brown covering that can be peeled off before usage. Aqueous/ethanolic extract of the seeds serves many medicinal usefulness. Such as purgative, antiparasitic and microbial actions (Iwu, 1993, Ogu and Ogu, 1995). Okwu (2005), has reported the nutritional and phytochemical contents of *A. melegueta* and *G. kola.*

Cymbopogon citracus (Lemon grass) is a member of a small group of aromatic grasses. It is a tropical tufted grass with long sharp-edged blades which usually grows in clumps up to 6 feet high (Kiefer, 2003). *C. citracus* is widely used as herbal remedies for menstrual pains and nausea (Simon *et al.,* 1984). It's antioxidant and sedative properties when consumed as tea, soup and curies has been noted (Ojo *et al.,* 2006). The plant has equally been reported to cure digestive disorders, rheumatism, head aches and relief muscle cramps and spasms (Gernot, 2001).

Osmotic fragility index is a measure of the resistance of the red blood cells to lysis by osmotic stress (Oyewale and Ajibade, 1990). Therefore, this present study seeks to evaluate the capacity of the red blood cells to withstand osmotic stress in the presence of aqueous extract of the three medicinal plants. This might give an insight into the property of these plant extracts to promote red blood cells membrane integrity or disintegration.

MATERIALS AND METHODS

Collection of Plant Materials

The seeds of *G. kola* and *A. melegueta* and leaves of *C. citracus* were harvested from a local botanical garden at Umoziri – Inyishi, Ikeduru Local Government Area, Imo State, Nigeria between the months of June and August, 2006. The plant materials were identified and authenticated by Dr. C.I. Onuoha, Department of Plant and Biotechnology, Imo State University, Owerri, Nigeria.

Sample Preparation

The seeds of *A. melegueta,* thin sliced samples of *G. kola* and fresh leaves of *C. citracus* were washed with distilled water and dried in an oven at 50°C for 24hours. The samples were pulverized separately with ceramic mortar and pestle.

Extraction of Plant Materials

Aqueous extracts of the dried pulverized plant specimen were prepared by adding 10gramms of each separate sample to 100ml of distilled water. The suspension were allowed to stand for 24 hours at room temperature and filtered with Whatman No. 1 filter paper. The filtrates constituted the stock aqueous extracts of the plant specimen (10%.w/v) and serial dilutions were made to obtain corresponding aqueous extract concentrations in the order: 0.8%. 0.6%, 0.4% and 0.2%:(w/v).

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Determination of Human Red Blood Cells (HbAA) Osmotic Fragility

Red blood cells Osmotic fragility was determined based on the method described by Parpart *et al.,* (1947). The fraction of red blood cells lysed when suspended in varying concentrations of saline solution was determined spectrophotometrically.

A stock solution of buffered sodium chloride, osmotically equivalent to 100g/l NaCl was prepared as follows: NaCl (90g), Na_2HPO_4. $2H_2O$ (17.1g) and $NaH_2PO_4.2H_2O$ (2.43g) were dissolved in 1 litre of distilled water. Dilutions equivalent to 9.0, 7.5, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, 3.5, 3.0, 2.0 and 1.0g/l NaCl respectively were made. Each dilution had a final volume of 50ml. (Chikezie and Ibegbulem, 2004).

A 5.0ml of each saline solution (9.0 – 1.0g/l NaCl) were introduced into corresponding test tubes while 5.0ml of distilled water was added to the 13th test tube. A 0.5ml of a particular plant extract of a noted concentration was delivered into each of the given set of test tubes (1st – 13th). To each test tube, 0.05ml of well – mixed EDTA venous blood was added and mixed thoroughly by inverting the tubes several times. For the control experiment, the same set of procedure was repeated but devoid of added aqueous plant extracts. The suspensions were allowed to stand for 30minutes at room temperature, after which, the content was centrifuged for 5 minutes at 1200g.

Evaluation of Percentage Haemolysis and Stabilization of Red Blood Cells

The absorbance of the supernatants contained in test tubes 1 -13 was measured with aid of a spectrophotometer (Model 6400, Jenway) at maximum wavelength (λmax) of 540nm. The ratio of individual absorbance of (1st - 12th) test tubes to the 13th test tube were obtained and multiplied by a factor of 100. These values represent percentage of haemolysed red blood cells at each corresponding saline concentrations (9.0 – 1.0g/l).

The corresponding concentration of saline solution (NaCl g/l) that caused 50% lysis of red blood cells is the mean corpuscular fragility (MCF) value. The relative capacity of the aqueous extracts of the three medicinal plant to stabilize (% stabilization) the red blood cell is calculated as percentage of the ratio of the difference of test and control samples (Tietz, 1976; Chikezie and Ibegbulem, 2004)

Statistical Analysis

The data were analyzed by the use of students't-distribution test of significance as described by Pearson and Hartley (1966).

RESULTS

The results presented in Table 1 showed, blood samples containing aqueous extract of *A. melegueta, G. kola* and *C. citracus* (test samples) exhibited increased MCF values which were significantly different ($p<0.05$) when compared with the control sample (MCF value = 4.70 ± 1.7g/l).

Therefore, red blood cells suspended in an environment of aqueous extracts of the three medicinal plants showed enhanced capacity to withstand osmotic stress.

Table 1. Red Blood Cells (HbAA) Mean Corpuscular Fragility and Percentage Stability in the Presence of Aqueous Extracts of *A. melegueta, G. kola* and *C. citracus.*

Aqueous Extract Concentration(%)	A. melegueta MCF value (g/l)	Stabilization(%	G. kola MCF value(g/l)	Stabilization(%)	C. citracus MCF value(g/l)	Stabilization(%)
0.0 (control)	*4.70 ± 1.7*	*0.00*	*4.70 ± 1.7*	*0.00*	*4.70 ± 1.7*	*0.00*
0.2	**5.85±1.1**	**24.47**	**5.60±2.2**	**19.14**	**5.10±2.9**	**8.51**
0.4	**5.60±1.3**	**19.14**	**5.75±2.1**	**22.34**	**4.90±2.5**	**4.26**
0.6	**5.10±0.9**	**8.51**	**5.30±1.2**	**12.76**	**5.00±2.1**	**6.38**
0.8	**5.20±0.8**	**10.64**	**5.70±1.4**	**21.28**	**5.10±2.2**	**8.51**

MCF values are means of 3 determinations ± S.D.

A cursory look at Table 1 showed that aqueous extract of *G. kola* exhibited a relatively greatest tendency to stabilize red cell membrane (12.76% - 22.34%). However, aqeuos extract of *A. melegueta* at 0.2% concentration showed a single highest capacity to stabilize red cell membrane (MCF = 5.85±1.1g/l; stabilization(%) = 24.47). A 0.4% concentration of aqueous extract of *C. citracus* exhibited the lowest capacity to promote red blood cell membrane to withstand osmotic stress (MCF = 4.90±2.5g/l stabilization(%) = 4.26).

DISCUSSION

The mechanical stability and resilience of the erythrocyte comes from a partnership between the redox status (Murray, 2003) and underlying meshwork (membrane skeleton) of its plasma membrane (Lux, 1999). Reports have shown that oxidative damage of erythrocyte membrane is the primary cause of reduced capacity of the red blood cells to withstand mechanical and osmotic stress (Laurence *et al.,* 1997 and Mayes, 1983).

Studies have shown the capacity of numerous agents to retard and reduce haemolysis by promoting membrane stability when red blood cells are subjected to osmotic stress. Using human red blood cells, earlier experiments showed that ethanol-water extract of *G. kola* (Elekwa, 1996 and Iwu, 1993), aqueous extract of *Fagara Zantoxyloids* (Sofowara, 1975) and desmethyl chloropromazine (Dean and Schecter, 1978) caused increased capacity of red blood cells to resist osmotic lysis. In a similar vein, the present study showed the capacity of aqueous extracts of the three medicinal plants to increase the MCF value of HbAA red blood cells. The MCF values of the red blood cells at corresponding aqueous extract concentrations of the three medicinal plant (Table 1), were significantly different ($p<0.05$) when compared with MCF value of the control sample (4.70± 1.7g/l). However, the reported increased MCF values of the test samples (which was a reflection of the capacity of plant extracts to stabilize the red blood cell membrane) did not exhibit a defined proportionality with the corresponding concentrations of the medical plant extracts.

However, the stabilization effect of aqueous extracts of the three medical plants on erythrocyte membrane, could be connected with the relative high content of antioxidants and scavengers of free radicals present in these plant. Okwu (2005), reported that *A. melegueta* and *G. kola* are rich sources of phytonutrients such as flavonoids, phenolic compounds and vitamins. Biologically, flavonoids and other related compounds function as potent water soluble super antioxidant and scavengers of free radicals which prevent oxidative damage of cell membrane (Manimi *et al.,* 1994). Also, Simon *et al.,* (1984) noted α-tocopherol as the major

component of the essential oil present in *C. citracus*. The best defined physiologic role of α-tocopherol is its ability to act as an antioxidant to unsaturated fatty acyl moieties of lipid component of plasma membrane. Thus, this vitamin protects the red blood cells membrane against oxidative damage (Horwitt *et al.,* 1956). These are probably the underlying reasons why red blood cells suspended in aqueous extracts of these three medicinal plants exhibited relatively remarkable tendency to withstand lysis in hypotonic conditions.

CONCLUSION

The present *in vitro* investigation has established that aqueous extracts of *A. melegueta, G. kola* and *C. Citracus* contained molecular species that are capable of stabilizing erythrocyte membrane when the red blood cells are suspended in hypotonic solutions. However, the potency of these plant extracts as anti haemolytic agents should be subjected to further *in vivo* investigation.

REFERENCES

Agoha, R. C., 1974. Medicinal plant of Nigeria. Offset dicker Facuitcet Waskunden Natnurwenten Schoppen Nwtherlands pp. 22-158.

Chikezie, P. C. and Ibegbulem, C. O., 2004. Effect of quinine on osmotic fragility of HbAA Red Blood Cells of guinea pigs J. of Innov. Life Sci. 8: 5-8.

Dean, J. And Schechter, A. N., 1978. Sickle cell anemia: molecular and cellular basis of therapeutic approach. The New England J. of Med. 229: 755.

Elekwa, T., 1996. Effect of aqueous extracts of Garcina kola seed and Fagara zanthoxyloids roots on human erythrocyte membrane stability and AT Pase activity. Ph.D thesis, University of Port Harcourt, Port Harcourt, Nigeria pp. 105 – 106.

Gernot, K., 2001. *Cymbopogon citracus* (Lemon grass). Cambridge University Press. Cambridge. 2nd Edition.

Horwitt, M. K, Harvey, C.C, Duncan, C.D and Wilson, W.C 1956. Effect of limited tocopherol intake in man with relationship to erythrocyte haemolysis and lipid oxidations. Am. J of clin Nutr. *4.* 408 – 419.

Isaacs – Sodeye, W. A., 1973. Proceeding of the symposium on *Fagara* and the red blood cells, Unife Press, Nigeria. pp. 32.

Iwu, M. M., 1993. Handbook of African medicinal plants. CRC Press, Inc. Boca raton, Florida. Pp. 45 – 215.

Kiefer, L., 2003. Triple oaks nursery and herb garden: Lemon grass. http.//www. Tripole oaks. Com/artdioplayphp?id = 10.

Laurence, D. R, Benneth, P.N. and Brown, M., 1997. Clinical Pharmacology, Churchil Livingston 8th edition.

Lux, S. E., 1999. Dissecting the red cell membrane skeleton. Nature 281: 428 – 429.

Manimi, H, Konoshita, M, Fukuyama, Y, Koduma, M Yoshizawa, T, Sugiura, M, Nakagawa, T Nakagawa, K and Tago, H., 1994. Antioxidant xanthones from *Garcina Subelliptica, Phytochemistry* 41:533 – 629.

Mayes, P. A., 1983. Metabolism of Carbohydrates in: Harper's Review of Biochemistry D.W. Martin, P.A. Mayes and V.N. Rodwell (Eds). Lange Medical Publications. California. 19th Edition 15: 161 – 167.

Murray, R. K., 2003. Red and white blood cells In: Harper's Illustrated Biochemistry R.K. Murray, D.K. Granner, P.A. Mayes and V.W. Rodwell (Eds). Lange Medical Publications. California. 26th Edition 52: 613 – 614.

Ogu, E. O. and Ogu, R. C., 1995. A Comparism of some chemical properties of *Garcina Kola* and hops for assessment of *Garcina* brewing value. Bioresource Technology. *54:* 1 – 4.

Ojo, O. O., Kabutu, F. R, Bell, M. and Babayo, U., 2006. Inhibition of paracetamol induced oxidative stress in rats by extract of *Xymbopogon citracus (*green tea). African J. of Biotech. 5:12.

Okwu, D. E., 2005. Phytochemicals, vitamins and mineral contents of two Nigerian Medicinal plants. International J. of Mol. Med and Ad. Sci. 1 41:375 – 381.

Okwu, D. E. and Ekeke, O., 2003. Phytochemical screening and mineral compisition of chewing sticks in South Eastern. Nigeria. Global J. Pure and Applied Sci. 9: 235 – 238.

Oyewale, J. O. and Ajibade, H. A., 1990. The osmotic fragility of erythrocyte of turkey. Vet. Archi 60: 91 – 100.

Parpart, A. K., Lorenz, P. B. and Parpart, E. R., 1947. Osmotic resistance of human red blood cell. J. of Clin Invest. 26: 636.

Pearson, E. S. and Hartley, H.Q, 1966: Biometric Tables for statistians. Cambridge University. Press. London (3rd Edition). 116 – 117.

Simon, J. E, Cahdwick, A. F, Crackie, L. E., 1984. Herbs and herbal oils. African J. of Biotech. 2:6.

Soforawa, E. A., 1975 Isolation and characterization of anti – sickling agent from *Fagara Zanthoxloids* roots. *Lloydie* 38.169.

Soforawa, E. A and Isaacs – Sodeye, W. A., 1971 Reversal of sickling and crenation in erythrocytes by the root extract of *Fagara zanthoxyloids Lloydia 34:*383.

WHO, 1978. Resolution – Drug policies and management: medical plants: WHO document NO WHO 31/33.