Research Paper

***Afr. J. Traditional,* Complementary and Alternative Medicines**
www.africanethnomedicines.net

ISSN 0189-6016©2009

PRELIMINARY *IN VITRO* ANTISICKILNG PROPERTIES OF CRUDE JUICE EXTRACTS OF *PERSIA AMERICANA*, *CITRUS SINENSIS*, *CARICA PAPAYA* AND ***CIKLAVIT®***.

E.E. J Iweala[1*], F.O Uhegbu[2] and G.N Ogu[2]

[1]Department of Biological Sciences, Covenant University, Ota, Nigeria
[2]Department of Biochemistry, Abia State University, Uturu, Nigeria
Email; *emekaiweala2004@yahoo.co.uk,emekaiweala@hotmail.com*

Abstract

The antisickling properties of crude juice extracts of the edible portions of three commonly consumed tropical fruits namely *Persia americana*, *Citrus sinensis,* and *Carica papaya* were investigated *in vitro* alongside a new drug preparation called *Ciklavit*® that has antisickling activity. Four different solvent extracts of the crude juice of each fruit including aqueous, acidic, alkaline and alcoholic extracts were prepared and their antisickling effects on sickle cell trait (HbAS) and sickle cell disease (HbSS) blood samples checked alongside *Ciklavit*®. Blood samples were stabilized using normal saline and the antisickling effects were checked by counting the number of sickle cells remaining after incubation of the blood samples with the crude fruit extracts and *Ciklavit*® for twenty-four hours. The results showed that *Ciklavit*® produced a sustained reduction in the number of sickle cells in both HbAS and HbSS blood samples. Also the alkaline and alcoholic extracts of *P. americana* and *C. papaya* produced significant reduction in the number of sickle cells.

Key words: Antisickling, *Ciklavit*®, *Persia americana*, *Citrus sinensis, Carica papaya*

Introduction

Sickle cell disease is encountered in many parts of the world but is predominant in people of African, Mediterranean, Indian and Middle Eastern descent as well as blacks and Hispanics in the Caribbean and Central America (Bownas, 2000). The disease is a genetic disorder associated with the synthesis of abnormal hemoglobin as a result of point mutation in the gene coding for its beta chain resulting in the substitution of glutamic acid with valine (Koch et al., 2000). The affected hemoglobin expresses gross changes in its chemical and physical properties especially shape and oxygen-carrying capacity. The distorted sickle shape of the Hemoglobin slows down its mobility through blood vessels resulting in intensely painful blockage that prevent vital oxygen and nutrients in the blood from reaching target organs and tissues thereby impairing their functions (Campbell et al., 1999). This results in a number of health problems such as brain damage, thrombosis, liver damage etc. (Enang, 1992).

Individuals can either be homozygous for the sickle cell hemoglobin (HbSS) or heterozygous (HbAS). The HbAS individuals are termed carriers and do not usually exhibit the grave health conditions associated with the HbSS individuals except when they are exposed for a long duration to conditions that support sickling (Koch et al., 2000). Certain conditions including low oxygen concentration, low pH and high temperature could cause a decrease in solubility of deoxy HbSS, leading to its polymerization into fibers that distort its shape and function (sickling). This culminates in symptoms and complications associated with sickle cell diseases including joint pain, anemia, fever, paleness, shortness of breath and jaundice (Bownas, 2000)

Treatment of sickle cell disease has proved difficult and inefficient due to its genetic origin. Earlier therapies included use of liver-based extracts and diets, oxygen vasodilator, carbonic anhydrase inhibitors and spleenectomy. Other conventional therapeutic management involves the use of drugs such as piracetam, tucaresol, hydroxyurea as well as bone marrow transplantation and gene therapy (el-Hazmi et al., 1995; Krishnamurti et al., 2001,Walters, 2005, Bownas, 2000, Dean and Schechter,1978). Research is still going on to discover potential antisickling agents, especially from plants (Sofowora et al., 1975, Adesina, 2005). The antisickling properties of plants are due to their content of alkaloids, resins, phenolics and oils, (Ekong et al., 1975, Elujoba and Nagels, 1985, Sofowora et al., 1975, Ouattara et al., 2004).

Fruits such as *Persia americana* (Avocado pear); *Citrus sinensis* (Orange), and *Carica papaya* (Paw-paw) are commonly consumed in the tropics and have a wide array of biologically active substances which could possibly show antisickling properties comparable to that of a new drug preparation called *Ciklavit*® (Pizzorno and Murray, 1985, Ahmed and Bonner 1980, Daziel, 1955, Bean, 1958, Cummings and Schroeder, 1942,). *Ciklavit*® produced from extracts of *Cajanus cajan* has been shown to reverse sickling and clinically reduce the painful crises associated with sickle cell disease (Ogoda et al., 2002, Akunsulie et al., 2005).The possible benefits that can accrue from new therapies for sickle cell disease from local African fruits in terms of safety and affordability when compared to conventional therapies such as hydroxyurea cannot be overemphasized.

Materials and Methods

The edible portion of matured and ripe fruits of *Persia americana* , *Citrus sinensis*, and *Carica papaya* bought from a local market were used. Blood samples from consenting and confirmed HbAS and HbSS volunteers attending the University of Nigeria Teaching Hospital (UNTH), Enugu, Nigeria were used.

Each (100g) of the washed and peeled fruits was extracted in 100mls of water using a juice extractor. The crude paste obtained was then extracted with different solvents.

Each fruit juice (10mls) was extracted with 5mls of 0.1N HCl by gently shaking the mixture for 1 hr in a shaker. The mixture was then centrifuged for 5 mins at 5,000g and the supernatant was concentrated to 5mls at 60^0C in a shaking water bath and retained as the acidic extract.

Each fruit juice (10mls) was extracted with 5mls of 0.1M NaOH by shaking the mixture gently for 1 hr in a shaker. The mixture was then centrifuged for 5 mins at 5, 000g and the supernatant concentrated to 5mls at 60^0C in a shaking water bath and retained as the alkaline extract.

Each fruit juice (10mls) was extracted with 5mls of 95% ethanol by shaking the mixture and they were then centrifuged for 5 mins at 5,000g and the supernatant concentrated to 5mls at 60^0C in a shaking water bath and retained as the alcoholic extract.

Each fruit juice (10mls) was extracted with 5mls of water by shaking gently for 1 hr in a shaker. The mixture was centrifuged for 5 mins at 5,000g and the supernatant concentrated to 5mls at 60^0C in a shaking water bath and retained as the water extract.

Antisickling tests

Antisickling test were done as outlined by Elekwa et al., (2003). Blood samples of HbAA, HbAS and HbSS patients were used to check for sickling on grease-free slides. Each blood sample (0.2mls) and 0.2ml of 2% sodium metabisuphite solution were mixed on slides, covered and rimmed with molten wax. The slides were incubated for 24 hrs and viewed under x40 magnification with a binocular light microscope (Olympus model CHC-XS2-107BN) for sickling after which sickle cells were seen in HbAS and HbSS samples. The antisickling effects were investigated by incubating 0.2mls of the acidic, alkaline, alcoholic and water extracts of each of the fruit juice extracts and 0.2mls of *Ciklavit*® solution on covered slides rimmed with molten wax for 24 hrs. The control consisted of only HbAS and HbSS blood samples and 2% sodium metabisulphite solution. All the tests were done in triplicates. After incubation, the numbers of sickled cells remaining in all the tests were counted under a light microscope.

Statistical analysis

The student t-test was used and statistical significance was checked at 95% confidence interval.

Results

Results obtained were expressed as percentage of sickle cells remaining after incubation of HbAS and HbSS blood samples with the different fruit extracts and *Ciklavit*®, as shown in Tables 1- 3.

Effect of *Citrus sinensis* on sickling

The effects of extracts of *Citrus sinensis* and *Ciklavit*® on sickling are shown in Table 1.The result indicates that *Ciklavit*® produced remarkable antisickling on HbAS and HbSS blood samples. Only the alkaline extract of *C. sinensis* showed a significant antisickling effect on HbAS blood sample.

Table 1: Antisickling effect of *c. sinensis* and ***ciklavit*®** on hbas and hbss blood

S/n	Test	Number of sickle cells (%)
1	HbAS blood alone	52
2	HbAS blood + Ciklavit	10*
3	HbAS blood + water extract	58
4	HbAS blood + Acidic extract	38
5	HbAS blood + Alkaline extract	15*
6	HbAS blood + Alcoholic extract	90
7	HbSS blood alone	92
8	HbSS blood + Ciklavit	25*
9	HbSS blood + water extract	88
10	HbSS blood + alkaline extract	45
11	HbSS blood + Acidic extract	44
12	HbSS blood + alcoholic extract	80

*****P≤0.05,n=3

Effect of *Carica papaya* on sickling

The antisickling effects of *Ciklavit*® and the extracts of *Carica papaya* on HbAS and HbSS blood are shown in Table 2. From the results, *Ciklavit*® produced a significant antisickling effects on both blood samples followed by the acidic ,alcoholic and alkaline extracts of *C. papaya* on HbAS and HbSS blood samples respectively.

Table 2: Antisickling effect of *c. papaya* and ***ciklavit*®** on hbas and hbss blood.

S/n	Test	Number of sickle cells (%)
1	HbAS blood alone	60
2	HbAS blood + Ciklavit	11*
3	HbAS blood + water extract	54
4	HbAS blood + Acidic extract	60
5	HbAS blood + Alkaline extract	55
6	HbAS blood + Alcoholic extract	15*
7	HbSS blood alone	85
8	HbSS blood + Ciklavit	24*
9	HbSS blood + water extract	53
10	HbSS blood + Acidic extract	58
11	HbSS blood + alkaline extract	28*
12	HbSS blood + alcoholic extract	48

*****P≤0.05,n=3

Effect of *Persia americana* on sickling.

The effects of *Persia americana* and *Ciklavit*® on sickling in HbSS and HbAS are shown in Table 3. Results showed that *Ciklavit*® produced the highest level of antisickling effect, followed by the alkaline and alcoholic extracts of *P. americana* on HbAS and HbSS blood samples respectively.

Table 3: Antisickling of *p. americana* and ***ciklavit*®** on hbas and hbss blood.

S/n	Test	Number of sickle cells (%)
1	HbAS blood alone	63
2	HbAS blood + ciklavit	11*
3	HbAS blood + water extract	90
4	HbAS blood + Acidic extract	31
5	HbAS blood + Alkaline extract	5*
6	HbAS blood + Alcoholic extract	84
7	HbSS blood alone	91
8	HbSS blood + Ciklavit	24*
9	HbSS blood + water extract	86
10	HbSS blood + Acidic extract	35
11	HbSS blood + alkaline extract	36
12	HbSS blood + alcoholic extract	28*

*****P≤0.05,n=3

Discussion

Sickle cell disease is a genetic disease that affects the hemoglobin causing it to assume a sickle shape that cannot effectively carry oxygen to tissues and organs. This abnormality results in crises and may manifest as symptoms including anemia, pain, fever, jaundice, leg ulcers etc (Koch et al., 2000). It has been difficult to find an efficacious cure for this disease because of its genetic origin, even though it can be managed by using some medications such as hydroxyurea (Bownas, 2000).

Some tropical plants have been found to have antisickling properties and are employed in the management of sickle cell disease (Sofowora, 1974, Sofowora et al., 1975, Elekwa et al., 2003. Only recently an indigenous pharmaceutical company in Nigeria produced a nutritional preparation called *Ciklavit*® from extracts of *Cajanus cajan* which purportedly has antisickling properties and is already being used in the management of sickle cell disease (Akunsulie et al., 2005). The antisickling potential of tropical plants is due to their peculiar content of a wide variety of biologically active substances and amino acids capable of reversing sickling (Sofowora, 1975, Ekeke and Shode, 1990)

The result obtained from this preliminary study confirms the *in vitro* antisickling property of *Ciklavit*® as well as the potential antisickling activity inherent in some tropical fruits such as *Persia americana*, *Citrus sinensis* and *Carica papaya*. These tropical fruits elaborate active constituents including essential oils, flavonoids, carotenoids and nitrogenous substances which could express antisickling activities in addition to other properties (Iwu, 1985,Thomas and Ajani, 1987, Duke, 1992, Eskin and Tamir , 2006, Ogunyemi et al., 2008, Elzebrook and Wind, 2008). Further investigations are underway to specifically characterize these potential antisickling substances from the fruits.

References

1. Adesina, S.K (2005). The Nigerian *Zanthoxylum* ; Chemical and Biological values. Afr. J. Trad., CAM **2(3):** 282-301.
2. Akinsulie, A.O, Temiye, E.O, Akanmu, A.S, Lesi, F.E.A and Whyte, C.O. (2005). *Clinical Evaluation of Extract of* Cajanus cajan *(Ciklavit®) in Sickle Cell Anaemia*. J Trop Pediatr. **51:** 200-205
3. Ahmed , E and Bonner, C (1980). Avocado, In Nagy's and Shaw (Eds): Tropical and Subtropical Fruits Composition, Properties and Uses. Avi Publishing West Point, Connecticutt, pp121-156.
4. Bean, R.C (1958). Changes in sugar during growth and storage of Avocados. California Avocado Society Yearbook **42**:90-93
5. Bownas, J. (2000). Genetic Profile: Sickle Cell Anemia. National Institutes of Health Publication, No. 96-4057.
6. Campbell, N.A, Mitchell, L.G and Reece, J.B (1999). Biology. Menlo Park, CA: Addison Wesley
7. Cummings, K and Schroeder, C. A. (1942). Anatomy of the avocado fruit. California Avocado Society Yearbook **27**:56-64.

8. Daziel J. (1955). The Useful Plants of West Tropical Africa Published by Crown Agents for Overseas Government and Administration, London. pp 52-63; 305-309; 310-316.
9. Dean, J. and Schechter, A. N. (1978). Sickle cell anemia: molecular and cellular basis of therapeutic approaches. New England J. Med. **229**:753-755.
10. Duke, J.A., (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants (and Database) Boca Raton, FL. CRC Press, Inc.
11. Ekeke, G. I. and Shode, F. O. (1990). Phenylalanine is the predominant antisickling agent in *Cajanus cajan* seed extract. Planta Medica **56**:41-43.
12. Ekong, D.E, Okogun, J.I, Enyhenihi,V.U, Balogh-Nair, B, Nakanishi, K and Natta, C (1975). ; New anti-sickling agent, 3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-butyric acid. Nature **258** (5537), 743-746.
13. Elekwa, I., Monanu, M. O. and Anosike, E. O. (2003). Effects of aqueous extracts of *Garcinia kola* seeds on membrane stability of HbAA, HbAS and HbSS human erythrocytes. Global J. Med. Sci. **2(2)**:97-101.
14. el-Hazmi M.A., al-Momen, A, Kandawammy, S, Haraib, S, Al-Mohreb, F. and Warsy, A.S (1995). On the use of hydroxyurea/erythropoietin combination therapy for sickle cell disease. Acta Haematol**, 94**(3): 128-134.
15. Elujoba, A.A. and Nagels, L. (1985). Chromatographic isolation and estimation of Zanthoxylum species. J. Pharm. Biomed. Analysis **3(5):** 447-451.
16. Elzebrook,A.T.G and Wind,K (2008). "Edible Fruits and Nuts" Guide to Cultivated Plants CABI pp:30-35.
17. Enang, U. (1992). Current concepts in the Management of Sickle Cell Disorder (A Practical Guide). Kraft Books Ltd. Ibadan, Nigeria Pp 41-45.
18. Eskin ,N.A.M and Tamir ,S (2006). "Avocado" Dictionary of Nutraceuticals and Functional Foods" CPR Press pp: 35-36
19. Iwu, M. M (1985) . Anti-hepatotoxic constituents of *Garcinia kola* seeds. Experientia **42**:699-700.
20. Koch, A.A, Yang, Q and Olney, R.S. (2000). Sickle hemoglobin allele and sickle cell disease: a HuGE review. Am. J Epidemiology, **151**(9): 839-845.
21. Krishnamurti, L, Blazar, B.R and Wagner, J.E (2001). Bone marrow transplantation without myeloablation for sickle cell disease. N Engl J Med., **344**:68.
22. Ogoda Onah.J, Akubue, P.I and Okide, G.B (2002). The kinetics of reversal of pre-sickled erythrocytes by the aqueous extract of Cajanus cajan seeds. Phytother Res.**16**(8):748-50.
23. Ogunyemi C.M, Elujoba A.A and Durosinmi M.A (2008). Antisickling Properties of *Carica papaya* Linn. J Natural Products, **1**:56-66
24. Ouattara, B., Angenot, L, Guissou, P, Fondu, P, Dubois, J, Frederich, M, Jansen, O, van Heugen, J.C Wauters, J.N and Tits, M (2004). LC/MS/NMR analysis of isomeric divanilloylquinic acids from the root bark of *Fagara zanthoxyloides* Lam. Phytochem. **65 (8)**: 1145-1151.
25. Pizzorno, J.E., Murray, M.T., (1985). A textbook of natural medicine. John Bastyr College Publications, Seattle, Washington.
26. Sofowora, E.A. (1974). Recent Developments in Research into the Antisickling Properties of *Fagara zanthoxyloides* (Orin Ata). The J. Pharm. (Nigeria) **5:** 8-14.
27. Sofowora, E.A, Isaac-Sodeye, W .A and Ogunkoya, L .O (1975). African Medicinal Plants: Isolation and Characterization of an anti-sicking agents from *Fagara zanthoxyloides* root.*Lloydia* **34:**169-174
28. Sofowora, E. A. (1975). Isolation and characterization of an anti-sickling agent from *Fagara zanthoxyloides*. Lloydia **38**:169-171.
29. Thomas, K.D and Ajani, B., (1987). Antisickling agent in an extract of unripe pawpaw fruit. Trans. Roy Soc. Tro.l Med. Hyg, **81**:510-511.
30. Walters, M.C (2005). Stem Cell Therapy for Sickle Cell Disease: Transplantation and Gene Therapy. Hematology **Jan**; 66-73