ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (98-103), Month: October - December 2017, Available at: www.researchpublish.com

The Haemolytic Effect of Aqueous Extract of Carica Papaya

[1]OBIEKWE UCHE WINIFRED, [2]ALUMANAH EDWIN. O

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF NIGERIA NSUKKA

Abstract: **Medicinal plants are the sources of many important scientific drugs of the modern world. The leaves of *Carica Papaya* were collected from Nsukka towns. Phytochemical studies were carried out on the crude aqueous leaf extract (decotion) using standard phytochemical methods. Blood samples from normal haemoglobin genotype homozygotes (HbAA), sickle heterozygotes (Hb AS) and sickle homozygotes (HbSS) of ages between 20 and 30 years collected from the University of Nigeria Teaching Hospital (UNTH), Enugu, were used for the study. The haemolytic activity of *Carica Papaya* aqueous extract was investigated using the normal haemoglobin genotype, sickle heterozygotes and sickle haemozygotes at different incubation period and at the different concentrations of the plant extract. The study of the haemolytic activity of *Carica Papaya* plant extract on different Hb-genotypes at different incubation periods showed heamolysis (invitro) significantly higher ($p<0.05$) in the genotype HbSS when compared to Hb AS and Hb AA individuals. The heamolysis observed in the Hb AS is significantly higher than that of Hb AA. The result of the study showed that *Carica Papaya* causes haemolysis which was more evident in genotype 'SS' due to the fragility and instability of sickle haemoglobin.**

Keywords: **Haemolysis, Genotype, phytochemical, *Carica Papaya*.**

1. INTRODUCTION

Medicinal plants are the sources of many important scientific drugs of the modern world. Quinine from Cinchoma bark, Reserpine from Rauwofia root, Digotoxin from Digitalis leaf, Atropine from Belladomma root and leaf, Morphine from Opium Capsule, are just few examples of the innumerable modern scientific drugs that are prepared from medicinal plants (Sofowara 1982, Iwu, 1983, Iwu, 1993, Ajazuddin and Shailendra, 2010).

Phytochemical report by many researchers (Nair et al 1997, Pamplona and Roger, 2001, Distasi et al, Okafor et al 2009, Reza, et al 2015, Mutiu, et al 2015, Nyamukuru, et al 2017, Zafar, et al 2017), showed that most medicine contain active ingredients which are therapeutically important and potent in medicine such ingredients includes alkaloids, saponins, tannins, flavonoids, essential oils, resins, glycosides and anthocyanins, sterols, triterpenoids and others. Although much study has been done on some medicinal plants, the toxicity aspect is often neglected. There is a need to take note of the adverse effects of herbal medicine even though its potency is not disputable. This study is aimed among other things to investigate the haemolytic activity (if any) of the aqueous leaf extracts of *Carica Papaya* plant commonly used in African Folk Medicine. Due to high hospital bills and other miscellaneous factors, most Africans resort to treatment of ailment with herbal medicine either by patronizing herbal practitioners or preparing the herbs by themselves. There is a need to study the toxicological effects of these herbs (if any).

2. MATERIALS AND METHODS

Materials:

Blood samples from human subjects were used for this study. Three individuals (females) with haemoglobin genotype AA (Normal homozyotes), two individuals (females) with Haemoglobin Hb - Genotype AS (Sickle heterozygotes), two individuals (females) with haemoglobin genotype SS (Sickle homozygote) between the ages 20-30 years were used for

this study. Blood specimen, 5.0ml were collected from each human subject with different haemoglobin genotypes HbAA, Hb AS and Hb SS and used for the research.

Material (Leaf Extract):

Carica Papaya (Family Meliaceae) leaves were collected from Nsukka town (Eastern Nigeria). The crude aqueous extracts (decotions) of these medicinal plants as being used by herbal practitioners were used since this is a toxicological study.

Methods:-

Preparation of medicinal plant extracts:

A decotion (herbal dose obtained by boiling of part of plants) of the medicinal plant *Carica Papaya* were prepared by boiling 175g of the leaf in 500ml of water. Thoroughly washed leaves of *Carica Papaya* were placed in a clean heat resistant container and 500ml of water was added. The boiling lasted for about 10 – 15 minutes on low heat. The resulting liquid was filtered through strain and used for the study.

Methods For Haematological Studies:

Genotype determination (Dacie and Lewis, 1999):

Genotypes were determined using cellulose acetate electrophoresis for separation of haemoglobins.

Principle: Haemoglobin when placed in an electric field will migrate to one of the electrodes. The difference in charge distribution and molecular weight of the haemoglobin at different pH Value is used in the separation of the haemoglobin.

Study of haemolytic activity of plant extract *Carica Papaya* (Spirichev, et al, 1989):

The method is based on the measurement of light absorption of exoerythrocytic haemoglobin. This test was carried out on all the subjects with the plants extracts at incubation period of 2 hours, 12 hours, 24 hours and 48 hours.

The plant extract was serially diluted and the dilutions of ½, 1/6, and 1/64 were also used to study the haemolytic activity of *Carica Papaya* at incubation period of 2 hours.

Calculation was carried out from the equation from the equation

$X=E1(100)/E3$

Where x = the degree of heamolysis caused by the plant extract

Where E1 = The absorbance measured for plant extract containing sample

Where E3 = The absorbance measured for control sample.

Phytochemical analysis of *Carica Papaya* plant extract.

Standard phytochemical methods (Harbourne, 2000) were used to test for the presence of alkaloids, flavonoids, glycosides, cardiac glycosides, cyanogenic glycosides, anthracene glycocides, proteins, carbohydrates, reducing sugars, saponins, steroidal aglycine, anthraquinone, tannins, lead sub acetate and ferric chloride.

TLC Techniques:

The TLC method was used for the detection of the various secondary metabolites in the crude extract of *Carica Papaya* (Fresh Leaves). The samples of the plant extract were spotted 2cm from the base of the plate using a capillary tube and allowed to dry before developing in appropriate solvent system in chromatographic tank.

Calculation of Rf:

Rf= The distance moved by a spot relative to the solvent front. Specific Rf Values were calculated using the formula

$Rf = DC/DS$

Where, DC = Distance moved by components

DS = Distance moved by solvent Fronts

Data Analysis:

Data were analyzed with computer using the SPSS version 7.5 software packages. Mean values (SD) experiments with duplicate samplings were taken for analysis. Differences between groups were assessed by one-way Anova while differences within were assessed by student t-test. The acceptance level of significance was $p<0.05$

3. RESULTS

Phytochemical characteristics of plant extract. *Carica Papaya* using chemical methods:

The result of the phytochemical screening of *Carica Papaya* (using chemical methods) showed the following constituents. Aqueous extracts of fresh leaves of *Carica Papaya* was found to contain reducing sugars, flavonoids, saponins, anthroquinones, tannins and steroidal aglycone.

The result of phytochemical screening (TLC method) of *Carica Papaya* plant extract:

The extracts of *Carica Papaya* (fresh leaves) showed presence of flavonoids, Terpenoids, saponins, sugars (Rhamnose, Xylose), Alkaloids, flavonoids, Cardiac glycoside, Terpenoids and Saponins.

The haemolytic effect of the *Carica Papaya* plant extract on Hb AA, Hb AS and Hb SS erythrocytes at different incubation periods of 2, 12, 24 and 48hours:

The study of haemolytic activity of *Carica Papaya* plant extract on different Hb-genotypes at different incubation periods showed heamolysis (invitro) significantly higher ($p<0.05$) in the genotype Hb-SS when compared to that of Hb AS and Hb AA individuals. The Haemolysis observed in Hb AS is insignificantly higher ($p>0.05$) than that of the Hb AA individuals. Generally, there was a significant increase ($p<0.05$) in the haemolytic activity as the incubation period increases from 2 hours to 48 hours for all the Hb-genotypes study.

The haemolytic effect of the various concentration of *Carica Papaya* plant extracts on Hb AA, Hb AS and Hb SS erythrocytes after 2 hours incubation periods:

The study of haemolytic activity of *Carica Papaya* plant extract on different Hb genotypes at different concentrations after an incubation period of 2hours showed a significant decrease ($p<0.05$) haemolysis (invitro) as the concentration decreased from ½ to 1/64 for all the Hb-genotypes. The haemolysis observed for the Hb-Genotype SS for all the concentrations of plant extract was significantly higher ($p<0.05$) when compared with Hb AA and Hb AS individuals.

4. DISCUSSION

The result of the phytochemical studies showed constituents which agrees with the report of other researchers who have done phytochemical studies on *Carica Papaya* (Oliver- Beever, 1982, Weniger et al, 1988, Nair, et al 1997, Pamplona and Roger, 2000, Distasi et al, 2002), however none of them reported the presence of Tannins as seen in this study. It is a known fact that the constituents isolated in plants are a function of the solvent used and the location of the plant.

The extract of *Carica Papaya* showed haemolytic activities at different concentrations and at different incubation periods which agrees with the study done earlier by Nohl and Klanu, (1998). The haemolysis observed in Hb Genotype SS is significantly higher ($p<0.05$) when compared to Hb AS and Hb AA erythrocytes. These results will be attributed to the fact that the sickle erythrocytes are more fragile than normal ones (Tse and Lux, 1999, Tanner and Anstel 1999, Hebbel, 1990). Hence there is accelerated auto-oxidation and heme loss due to instability of sickle haemoglobin (Hebbel et al, 1988).

5. CONCLUSION

The results of this studies showed that *Carica Papaya* plant extract has haemolytic effect (invitro) on all the haemoglobin genotypes, being significantly higher, ($p<0.05$) in Hb SS due to the fragility and instability of sickle haemoglobin. This folk medicine therefore should be taken in low doses and with caution.

ACKNOWLEDGEMENT

My profound gratitude to the Almighty God who made it possible for me to carry out this research work. My unfeigned gratitude to my supervisor Prof. E.O. Alumunah, for his fatherly disposition towards me, encouragement, prompt response

to problems and above all being ever ready to attend to me. My immense gratitude to my beloved mother Mrs. M.N.Obiekwe and other members of my wonderful family. I wish to appreciate all the lecturers and members of staff of the department of Biochemistry, UNN and my colleagues for various roles played in course of this work. My gratitude to management and staff of BDCP, Nsukka, entire staff of safety laboratory, Nsukka and Sickle cell club of UNTH, Enugu for their co-operation and support. I wish to appreciate everyone who in one way or the other contributed to the success of this work; God will surely reward your labour of love.

REFERENCES

[1] Ajazuddin .A. and Shailendra Saraf (2010). Evaluation of Physiochemicial properties of Safoof .E. Sana, a Unani Polyherbal formulation. Pharmacognosy Research Year 2010, Vol 2, Issue 5, pp 318-322.

[2] Distasi, L.C., Oliveira, G.P, Cariralhaes, M., Queirez, J., Tien, S.O., Kakinami, S.H. and Reis, M.S. (2002). Phytochemical screening of some tropical plants. J. for the study of medicinal plants 73 (1): 78-81.

[3] Harbone, J.B. (2000) Phytochemical method. A guide to modern techniques of plant analysis. Chapman and Hall, London. Pp 228-276.

[4] Hebbel R.F. (1990). The sickle erythrocyte in double jeopardy. Autoxidation and iron decompartmentalisation. Seminar in haematology. 27:51-69.

[5] Iwu, M.M. (1983). Traditional Igbo Medicine. Inst. Of African studies proj. UNN.

[6] Iwu, M.M. (1993). Handbook of African Medicinal Plants. CKC Press, London. Pp 3, 124-128.

[7] Mutiu Idowu Kazeem, Anofi Omotayo and Tom Ashafa (2015). In-vitro antioxidant and antidiabetic potential of Dianthus Basuticus Burtt Davy whole plant extract. Journal of Herbal Medicine Vol 5. Issue 3. (May 2015) Pp 158-164.

[8] Nair, M.S., Gopal, S. and Isaac, O. (1997). Optimised isolation procedures for biologically active compounds, nimbolide and 28 deoxonimbolide. J.Phytochemical 46 (1):117.

[9] Nyamukuru Anthonia, Fabula John, Lamonda Mohammad, Kato Bernard, Sekagya Yahaya and Aduma Philip (2017) Medicinal plants and Traditional treatment practices used in the management of HIV/AIDS Clients in Mpigi Districts Uganda Journal of Herbal Medicine Vol 17. Pp 51-58.

[10] Nohl, H. and Klanu, S. (1998). The effect of Xenobiotics on erythrocyte. General pharmacosy 31 (3): 343-347.

[11] Oliver-Bever, B. (1986). Medicinal Plants of tropical West Africa. Cambridge University Press London. P.375.

[12] Okafor, G.I, Okoli, C.O, Odo, AS. and Kelechi, N.R. (2009). Studies on the effects of processing methods on the Antihyperglycemic activity of herbal teas. Pharmacognosy research year 2009, Volume I, Issue 5. Pp 256-260.

[13] Pamplona, G.D. and Roger, M.D. (2001)a. Encyclopedia of medicinal plant education and health library I. Safeliz – Spain Pp. 694 - 701.

[14] Reza Mahdavi, Javad Heshman and Wazil Namazi (2015). Effect of black seeds (Nigella Sativa) on male fertility. A systematic review article. Journal of Herbal Medicine Vol 5: Issue 3. Pp 133 – 139.

[15] Spirichev, V.B. and Blazeheyerich, N.B. (1989). Study of haemolytic action of vitamin D. In: Manual in Biochemistry. Stroev, E.A. and Makarova, V.G. (eds). Mir Publishers Moscow, Russia. Pp 251 – 252.

[16] Sofowara, A. (1982). Medical Plants and Traditional medicine in Africa. John Wiley and Sons Ltd, New York. Pp. 20 – 21.

[17] Vanwyk, B.E. and Nigel, G. (2000). People's plant. A guide to useful plants of southern Africa. Briza Publications, Pretoria South Africa. Pp 230 – 246.

[18] Weniger, B. and Robineau. L. (1988). Elements for Caribean Pharmacopicea. Proceedings of Tramail 3 Workshop, Cuba. November, 1988.

[19] Zafar Igbal, Muhammad Asuf, Naveed Aslam, Naveed Akhtar, Muhd Zainia, Aswam Yam, Mun Tei and Qaiser Jabeen (2017) clinical investigation on gastroprotective effects of ethanolic extract of phyllanthm cmibica Linn Fruit. Journal of Herbal Medicine (March 2017) Pp 11 – 17.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (98-103), Month: October - December 2017, Available at: www.researchpublish.com

APPENDIX - A

Table 1

Table 1: Phytochemical screening of medicinal plants (Chemical methods)

TESTS	PLANT EXTRACT
Biuret test	Nil
Millons test	Nil
Reducing sugar test	+ve
Flavonoids with $ALCl_3$	+ve
Flavanoids with dil Ammonia	+ve
Saponins – Emulsion	+ve
Saponins Stable	+ve
Alkaloids (Mayer's reagent)	Nil
Alkaloids (Wagner's reagent)	+ve
Alkaloids (Picric acid)	Nil
Alkaloids (Draugendroff's reagent)	+ve
Anthraquinone	+Ve
Tannins with lead sub acetate solution	+ve
Tannins with Ferric chloride	+ve
Molish test	+ve
Anthracene glucoside	Nil
Steroidal aglycone	+ve
Cardiac glycosides	Nil
Cyanogenic glycosides with Fehling's solution	Nil
Cyanogenic glycosides with dil H_2SO_4	Nil

Table 2

Result of osmotic fragility test on AA, AS and SS individuals using NaCl buffer and *Carica papaya* plant extract

GENO-TYPE	TUBE	1	2	3	4	5	6	7	8	9	10	11	12 (Distrilled water)
	Vol (ml) stock NaCl soln (10g/l	4.5	3.8	3.3	3.0	2.8	2.5	2.0	1.8	1.5	1.0	0.5	
	Vol (ml) of plant extract	0.5	1.2	3.7	2.0	2.2	2.5	3.0	3.2	3.5	4.0	4.5	
	Final conc.of NaCl(g/l)	9.0	7.5	6.5	6.0	5.5	5.0	4.0	3.5	3.0	2.0	1.0	
	Vol. Of blood (ul)	50	50	50	50	50	50	50	50	50	50	50	
AA	%lysis	0	0	0	0	6.7±0.45	18.0±0.55	26.4±2.17	35.4±0.74	37.8±1.91	40.3±1.77	47.9±1.39	
AS	%lysis	0	0	0	0	17.7±1.41	21.6±0.64	28.2±0.92	37.6±0.28	40.9±1.27	46.5±1.20	54.7±0.35	
SS	%lysis	0	0	5.7±2.12	18.4±0.07	22.4±2.26	35.0±7.07	42.0±1.70	44.5±0.42	54.3±0.14	55.14±0.24	62.0±0.57	

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (98-103), Month: October - December 2017, Available at: www.researchpublish.com

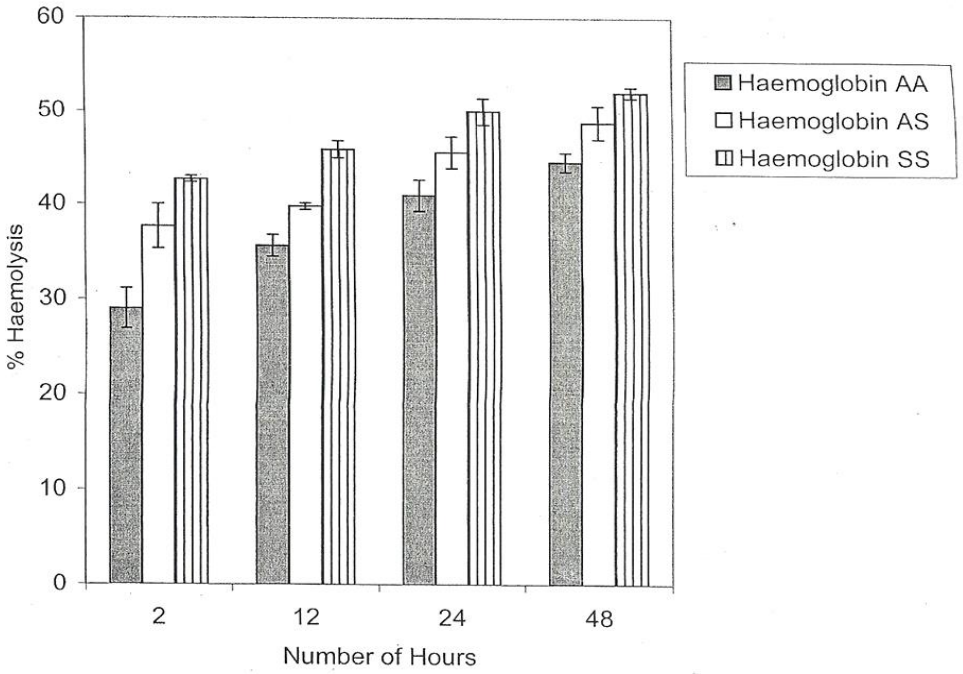

Fig. 1 : HISTOGRAM OF HAEMOLYTIC ACTIVITY OF CARICA PAPAYA PLANT EXTRACT ON DIFFERENT HAEMOGLOBIN GENOTYPES AA, AS AND SS AT DIFFERENT INCUBATION PERIODS OF TWO, TWELVE, TWENTY-FOUR AND FORTY-EIGHT HOURS.

Figure 1

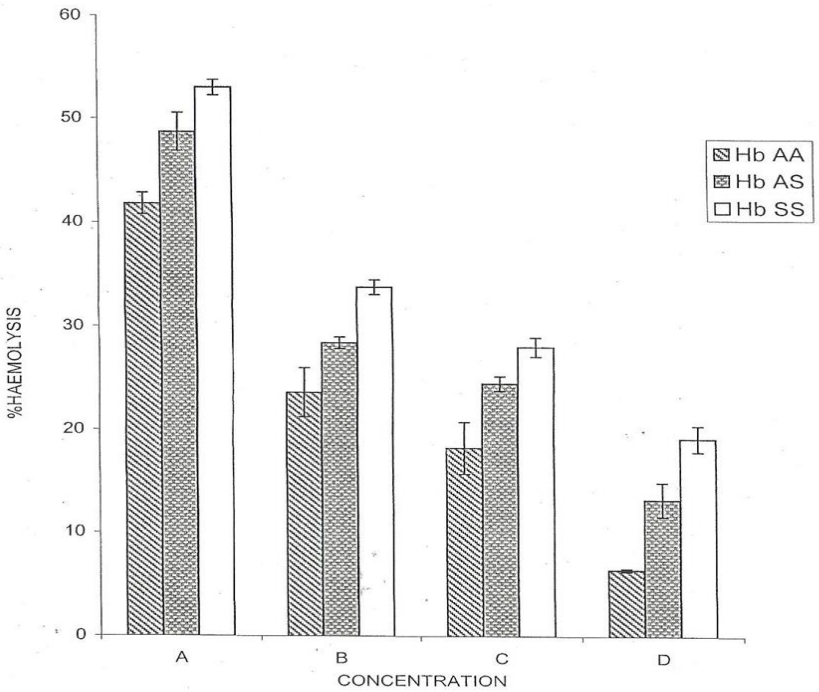

Fig. 2: HISTOGRAM OF HAEMOLYTIC ACTIVITY OF C.PAPAYA PLANT EXTRACT ON THE DIFFERENT Hb GENOTYPES AA,AS,AND SS AT DIFFERENT CONCENTRATIONS A,B(1/2),C(1/16),D(1/64) AT INCUBATION PERIOD OF 2HRS.

Figure 2