ISSN 1996-3351

Asian Journal of Biological Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Biological Sciences

ISSN 1996-3351
DOI: 10.3923/ajbs.2020.134.138

Research Article
Biokinetics and Phyto-toxicity of Pumpkin Leaves Extract on Human Erythrocytes, "An *in vitro* Study"

[1]N.I. Ibeh, [2]M.A. Okungbowa, [3]T. Ekrakena and [2]I.N. Ibeh

[1]Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria
[2]Department of Medical Laboratory Sciences, Faculty of Basic Medical Sciences, University of Benin, Benin City, Nigeria
[3]Department of Life Sciences, Faculty of Basic Sciences, Benson Idahosa University, Benin City, Nigeria

Abstract

Background and Objectives: Pumpkin leaves (*Telfairia occidentalis*) are extensively used in homes in Nigeria and some West African countries for soup making and sometimes extracted in water for drinking as medicament for stimulating erythropoiesis. The present study was aimed at evaluating the *in vitro* effect of pumpkin leaves extract on human erythrocytes with an interest in obtaining base data on the phyto-toxicity. **Materials and Methods:** Pumpkin leaves weighing 250 g was extracted in 250 mL cold distilled water to obtain a stock solution of 1 g mL^{-1} pumpkin leaves extract. The stock solution was further diluted in sterile test tubes to obtain a concentration range of 10, 20, 40 and 640 µg m^{-1} by using cold sterile distilled water. The test required the addition of 0.5 mL of each pumpkin leaves extracted concentration to a sterile test tube containing 2.5 mL of 5% human erythrocyte in sterile physiological saline and allowed to remain at room temperature (27+1°C) for 30 min. The hemolysis occurring in the tubes was read spectrophotometrically (Cornings) at 540 nm wavelength along with the neat and saline control. **Results:** The absorbance reading reflected corresponding increase in the concentration of pumpkin leaves extract by using phosphate. This showed a significant increase in toxicity *in vitro* as the concentration gradient was increased, the least concentration with stability of the erythrocyte *in vitro* as compared with the phosphate buffer is termed an equivalent for the determination of toxicity in erythrocyte *in vitro-in vivo* by other phyto products. **Conclusion:** The data obtained from this study has value in determining phytotoxicity with pumpkin leaves and any other phyto-products on human erythrocytes with all its determining variables in consideration.

Key words: *In vitro-In vivo*, hemolysis, phyto-toxicity, erythrocytes, *Telfaira occidentalis*, spectrophotometrically, biokinetics, triton-x

Citation: N.I. Ibeh, M.A. Okungbowa, T. Ekrakena and I.N. Ibeh, 2020. Biokinetics and phyto-toxicity of pumpkin leaves extract on human erythrocytes, "An *in vitro* study". Asian J. Biol. Sci., 13: 134-138.

Corresponding Author: N.I. Ibeh, Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Benin, Benin City, Nigeria

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biomaterials are either derived from nature or synthesized by using polymers, ceramics, metals and composite materials. Specifically, biomaterials have been extensively applied to controlled release systems since 1976 that continually released macro-molecules to inhibit angiogenesis[1]. There have been many studies on drug delivery[1], but *in vitro* toxicity assessments a carrier must be developed. Most reports have assessed cytotoxicity by using only target cells or non-specific cells from animals. This approach cannot represent the overall toxicity for humans because of the differences between many of the cells used in these studies and human cells[2].

Charles Telfair (1778-1835), an Irish botanist who lived in Mauritius, sent an African genus of the cucumber family (Cucurbitaceae) from Mauritius to Sir Williams Jackson Hooker (1785-1865) for identification. In honor of Dr. Telfair, the plant *Telfairia occidentalis* was named after him[2]. However, the earliest reference to *Telfairia* was made by Oliver in 1871 and it recorded its presence in Upper Guinea areas of Sierra Leone, Fernando Po and Abeokuta (Nigeria)[3-5].

Telferia occidentalis which belongs to the family Cucurbitaceae is one of the leafy vegetables widely consumed in Nigeria for its nutritional and therapeutic benefits. It is widely cultivated in the south-eastern, south-western and south-southern parts of Nigeria and utilized in the preparation of soups and medicines. Dietary intake of *Telferia occidentalis* could reduce garlic induced hemolysis in rats[3,4]. The aqueous extracts of *Telferia occidentalis* has been found to reduce blood glucose levels and also have anti-diabetic effects in glucose induced hyperglycemic and streptozotocin induced diabetic mice[5,6]. Also, aqueous extracts of *Telferia occidentalis* has been reported to assist in the purging of the gastro-intestinal tract as revealed by the purgative effects of the aqueous extracts of *Telferia occidentalis* leaf on isolated Guinea pig ileum[7-10].

The leaves are rich in vitamins and minerals such as; Ca, P and Fe etc. The seed is also eaten as food. The oil obtained from the seed is used in cooking[4,5,10]. *Telfairia occidentalis* has been reported to possess antioxidant property. The aqueous extract had a high total phenol, reducing power and free radical scavenging ability (12.2%, 1.9 OD700 and 92%, respectively) than the ethanolic extract which had total phenol, reducing power and free radical scavenging ability of 5.5%, 1.5 OD700 and 25%, respectively[9,10]. The free soluble polyphenols content in the leaf of the plant which was higher than the bound polyphenols had higher antioxidant activity as typified by their higher reducing power and free radical scavenging ability than the bound polyphenols[6,7]. *Telfairia occidentalis* leaf contained a significantly high amount of vitamin C, total flavonoids and phenolics than *Psidium guajava* stem bark. The leaf inhibited more free radicals than *Psidium guajava* stem bark[10,11]. The n-hexane fraction had the highest flavonoid content and free radical scavenging activity comparable to that of the commercial antioxidant[12,13]. The ability of the leaf of *Telfairia occidentalis* to reduce iron (III) to iron (II) was also reported[13]. The antioxidant property of *Telfairia occidentalis* is attributable to the high content of polyphenols, especially flavonoids. In the search for methods to evaluate the toxicological risk of chemicals without employing animal experimentation, much emphasis has been put on the replacement of acute toxicity (LD_{50}) determinations. One important assumption was that acute toxicity is related to a compound's basal cytotoxicity[13]. The erythrocytes of humans and mammals represent a good model to evaluate the cytotoxicity of molecules, organic and inorganic, natural or synthetic by cellular damage measure[14-16]. Indeed, before any investigation on the mechanism of action of different molecules, it is important to perform a cytotoxicity assay[7]. Among the different cytotoxicity assays that assess a possible toxicity in the red blood cells is the rate of hemolysis[7]. This essay is based on the evaluation of the alterations of red cell membranes in the presence of an eventual xenobiotic[17,18]. Red blood cells are the main cells in circulation and they are responsible for transporting oxygen; in fact, any alterations of this process could be lethal. The present study was aimed at evaluating the *in vitro* effect of pumpkin leaves extract on human erythrocytes with an interest in obtaining base data on the phyto-toxicity.

MATERIALS AND METHODS

Study area: This study was carried out between the month of March and September, 2018 in the University of Benin, Health Services Department, Laboratory Unit, University of Benin and the Laboratory in the Department of Medical Laboratory Sciences, University of Benin, Nigeria.

Inclusion criteria: Red blood cell with the ABO blood group types from healthy patients to determine the *in vitro* phyto-toxicity of pumpkin leaves.

Exclusion criteria: Blood samples from patients who are on any form of medication which could interfere with the toxicity studies.

Ethical approval: Permission shall be sought and obtained from the Ministry of Health, Benin City, Edo state, Nigeria.

Sample collection

Blood sample: Arterial/Venous blood is collected aseptically from the cubital fossa and dispensed into a EDTA coagulated specimen bottles (5 mL) was deposited.

Methodology

Phyto-material extraction: The leaves of the plant were collected, dried in the shade and powdered using mortar and pestle. The powdered processed leaves were stored in airtight containers and labeled properly. Each of the dried grounded material weighed 500 g. Extraction of the phyto-product was carried out by using 2 L of methanol and normal saline by cold maceration for 7 days in large amber bottles with intermittent shaking. Filtration by using the Whatman filter paper (No. 42) was used to separate the artifacts and macro-substance.

Collection of blood and preparation for analysis: Using a sterile 5 mL syringe, 5 mL of blood was collected by veni-puncture from the cubital fossa of healthy patients without gender discrimination. The blood was dispensed into sodium EDTA specimen bottles (green cap), it was mixed gently and thoroughly rolling the bottle. Centrifugation was carried out to separate plasma from the packed erythrocytes. The separated packed erythrocytes were washed 3 times with phosphate buffered normal saline and the supernatant decanted. The time of centrifugation was 5 min at a speed of 626 (xg)[8,9]. The washed packed cells were used for the toxicity test by *in vitro* red cell hemolysis.

Haemolysis study: Hemolytic toxicity of pumpkin equivalent was checked by incubating the formulations with red blood cells separated from human blood by centrifugation at low speed and analyzing the samples for hemoglobin release at 541 nm. Hemolysis with different formulations were compared with that obtained with Triton-X100 as a positive[15].

Cell viability test: Hemolysis potentials of the pumpkin leave extract equivalent were added to the RBC concentrate and gently mixed. The concentrate was then incubated at 37°C for 30 min in incubator.

After incubation, it is again at 3000 rpm for 5 min to separate the pellet. The supernatant was analyzed for absorbance at 540 nm in UV spectrophotometer against normal saline as blank.

Percentage of hemolysis was determined for different samples considering the absorbance value of sample treated with 0.5% Triton-X100 to represent 100% hemolysis and normal saline treated samples to serve as negative control. Relative hemolysis (%) was determined by following expression[18]:

$$\text{Relative hemolysis (\%)} = \frac{\text{100\%-Abs sample-Abs negative}}{\text{Abs neg-Abs posi-100}}$$

About Table 1, a varied concentration the lethal dosage of pumpkin leaves extract was determined with doubling dilutions of 10^1-10^9 by using the heamolytic assay to determine cytotoxicity on human red blood cells. The absorbance values at 540 nm as measure of its toxicity.

At 125, 250, 500 and 1000 mg doses, respectively, the concentration gradient from 10^1-10^9, the effect of pumpkin leave extract determine its equivalent (X). A steady increase in absorbance values as the concentration of the pumpkin leave extract continued to increase exponential.

About Table 2, a varied concentration the lethal dosage of pumpkin leaves extract was determined with doubling dilutions of 10^1-10^9 by using the heamolytic assay to determine cytotoxicity on human red blood cells. The percentage haemolysis was determined by the formula:

$$\text{Heamolysis (\%)} = \frac{\text{Absorbance value of test-Absorbance value of PBS}}{\text{Absorbance value of triton X-absorbance values of PBS}} \times 100$$

Comparatively the percentage haemolysis at 125, 250, 500 and 1000 mg doses, respectively, is determined by comparing the positive and negative controls (Triton-X and PBS) against the concentration gradient from 10^1-10^9, of pumpkin leave extract and human red blood cells. Its equivalent (X) showed a steady increase in hemolysis values as the concentration of the pumpkin leave extract continued to increase exponentially.

Statistical analysis: Statistical analysis including descriptive statistics carried out by using the Statistical Package (Graph Pad Prism). All values will be expressed as Mean±SE (Mean standard error of mean). The Analysis of Variance (ANOVA) used to determine significant difference in test and control groups ($p<0.05$) at confidence limit will be set at 95%.

Table 1: Determination of lethal toxicity dosage of pumpkin extract on human red blood cells *in vitro* using a doubling dilution concentration vs. the absorbance value (Mean±SEM)

Dose (µg)	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9	p-value
125	0.3306±0.001	0.3304±0.002	0.3375±0.002	0.3394±0.002	0.3716±0.004	0.4034±0.002	0.4078±0.002	0.4955±0.002	0.5567±0.009	0.0001
250	0.3067±0.003	0.3124±0.002	0.3206±0.004	0.3394±0.003	0.3598±0.015	0.3953±0.005	0.4037±0.002	0.4184±0.002	0.4470±0.008	0.0001
500	0.4955±0.002	0.4975±0.001	0.5001±0.000	0.5019±0.001	0.6800±0.006	0.7600±0.005	0.8710±0.008	1.033±0.020	1.0780±0.0233	0.0001
1000	0.8599±0.005	0.8757±0.006	0.9731±0.001	1.033±0.020	1.048±0.021	1.078±0.023	1.133±0.003	1.773±0.064	2.0190±0.038	0.0001

Table 2: Comparing the mean absorbance and percentage hemolysis of *Telfaira occidentalis* leaves extract to determine its equivalent (X)

Dose	Absorbance (540 nm)	Hemolysis (%)
125 µg	0.3969	3.40
250 µg	0.3670	2.30
500 µg	0.7130	0.15
1000 µg	1.1991	32.90
PBS	0.3023	0.00
Triton-X	3.0200	100.00

RESULTS

The phytotoxic effect of pumpkin leaves equivalent was determined by carrying out a hemolytic assay on human red blood cells. About 5 mL of the various ABO blood group was collected from 292 healthy human subjects and dispensed into the EDTA bottle this selection was void of gender bias. *Telfaira occidentalis* were extracted at varied concentration, 125, 250, 500 and 1000 µg by using water as the solvent of extraction.

The pumpkin equivalent was determined comparing the least and the highest dose concentration gradient (125, 250, 500 and 1000 µg), there is a significant difference due to dose and concentration gradient, with 250 µg showed the closest when compared with phosphate buffered saline (2.3% hemolysis) (Table 1 and 2).

From the observations of this study, *Telfaira occidentalis* extract showed a dose dependent increase in heamolytic activity, there was low to mild heamolytic effect towards human erythrocyte, the dose of 125 µg showed an absorbance value of 0.3969 (3.4%) on the erythrocyte which was close to the absorbance value of phosphate buffered saline erythrocyte, 0.3023 (0%).

The pumpkin equivalent was determined to comparing the equivalent with phosphate buffered saline (0%) hemolysis and Trition-X (100%) hemolysis. The pumpkin equivalent closet to the phosphate buffered saline was at 250 µg with no significant difference across the ABO blood groups ($p>0.005$).

DISCUSSION

The *in vitro* effect of pumpkin leaves extract on human erythrocyte was determined comparing its hemolytic effects on human erythrocyte as a measure of its toxicity this led to the discovery of an equivalent lethal toxic concentration[12,13]. *Telfaira occidentalis* leaves extract showed significant less toxic effect *in vitro* at room temperature when compared with *Ocimium gratissimum* and *Vernonia amygdalina* ($p<0.005$) there was similarity when compared with works carried out on the stabilizing effect of pumkin leave extract on sickle cell erythrocyte[17].

All of the concentration of *Telfaira occidentalis* leave extract showed minimal heamolytic effect on human erythrocyte, 3.4, 2.3, 15 and 32.9%, respectively as compared with Triton-X and PBS solution, this coincides with previous studies which showed the stabilizing effect of *Telfaira Occidentalis* leave extract on human erythrocyte *in vitro*[10-12].

This study showed an elevation in the hemolytic effect on the human erythrocyte as the concentration increased which showed that at a higher concentration *Telfaira occidentalis* showed some level of toxicity on human erythrocyte conferring with the dose dependent toxicity this is synonymous with studies carried out by Foller *et al.*[13] who discovered a lethal concentration of *Telfaira occidentalis* at high concentration.

CONCLUSION

From the results obtained in this study *Telfiara occidentalis* has a potential as a tool for determining phyto-toxicity, this is due to its stabilizing effect on the erythrocyte at a lower concentration. The pumpkin leave equivalent will provide an alternative from by using laboratory animals for toxicity studies, but to replace them with erythrocyte *in vitro* as a measure *in vivo* for determine toxic concentration across a concentration gradient in any phyto-product. However, there is still need for further studies to evaluate its sensitivity, specificity and accuracy.

SIGNIFICANCE STATEMENT

This study has discovered a significant similarity with phytotoxicity studies done *in vitro* comparing with *in vivo* by using human erythrocyte as alternative instead of laboratory animals and bring to the fore front the use of cells as an alternative for laboratory animals in both one phase and multi-phase toxicity studies considering sequestration points and active sites.

ACKNOWLEDGMENT

Many thanks to the National Academy for The Advancement of Science for grants given by providing a working environment.

REFERENCES

1. Akoroda, M.O., 1990. Ethnobotany of *Telfairia occidentalis* (cucurbitaceae) among Igbos of Nigeria. Econ. Bot., 44: 29-39.
2. Baki, M.A., A. Khan, M.A.A. Al-Bari, A. Mosaddik, G. Sadik and K.A.M.S.H. Mondal, 2007. Sub-acute toxicological studies of pongamol isolated from *Pongamia pinnata*. Res. J. Med. Med. Sci., 2: 53-57.
3. Feres, C.A.O., R.C. Madalosso, O.A. Rocha, J.P.V. Leite, T.M.D.P. Guimaraes, V.P.P. Toledo and C.A. Tagliati, 2006. Acute and chronic toxicological studies of *Dimorphandra mollis* in experimental animals. J. Ethnopharmacol., 108: 450-456.
4. Nkereuwem, A.O., O.A. Eseyin, S.A. Udobre and A. Ebong, 2011. Evaluation of antioxidant activity and chemical analysis of the leaf of *Telfairia occidentalis*. Niger. J. Pharmaceut. Applied Sci. Res., 1: 21-28.
5. Nwanna, E.E. and G. Oboh, 2007. Antioxidant and hepatoprotective properties of polyphenol extracts from *Telfairia occidentalis* (Fluted Pumpkin) leaves on acetaminophen induced liver damage. Pak. J. Biol. Sci., 10: 2682-2687.
6. Oboh, G., E.E. Nwanna and C.A. Elusiyan, 2006. Antioxidant and antimicrobial properties of *Telfairia occidentalis* (Fluted pumpkin) leaf extracts. J. Pharmacol. Toxicol., 1: 167-175.
7. Verma, K.S. and Z. Baksh, 2013. Ethno-medicinal Plant Resources of Jabalpur district, Madhya Pradesh. J. Res. Dev., 12: 39-48.
8. Wan, J., W.D. Ristenpart and H.A. Stone, 2008. Dynamics of shear-induced ATP release from red blood cells. Proc. Natl. Acad. Sci. USA., 105: 16432-16437.
9. Whitton, P.A., A. Lau, A. Salisbury, J. Whitehouse and C.S. Evans, 2003. Kava lactones and the kava-kava controversy. Phytochemistry, 64: 673-679.
10. Wingstrand, K.G., 1956. Non-nucleated erythrocytes in a teleostean fish *Maurolicus mülleri* (Gmelin). Z. Zellforsch. Mikrosk. Anat., 45: 195-200.
11. Yazdanbakhsh, K., C. Lomas-Francis and M.E. Reid, 2000. Blood groups and diseases associated with inherited abnormalities of the red blood cell membrane. Transfusion Med. Rev., 14: 364-374.
12. Zhu, M., K.T. Lew and P. Leung, 2002. Protective effect of a plant formula on ethanol induced gastric lesions in rats. Phytother. Res., 16: 276-280.
13. Foller, M., S.M. Huber and F. Lang, 2008. Erythrocyte programmed cell death. IUBMB Life, 60: 661-668.
14. Garg, S.K., M.A. Shah, K.M. Garg, M.M. Farooqui and M. Sabir, 1997. Antilymphocytic and immunosuppressive effects of *Lantana camara* leaves in rats. Indian J. Exp. Biol., 35: 1315-1318.
15. Gesler, W.M., 1992. Therapeutic landscapes: Medical issues in light of the new cultural geography. Soc. Sci. Med., 34: 735-746.
16. Goodman, S.R., A. Kurdia, L. Ammann, D. Kakhniashvili and O. Daescu, 2007. The human red blood cell proteome and interactome. Exp. Biol. Med., 232: 1391-1408.
17. Gregory, T.R., 2001. The bigger the C-value, the larger the cell: Genome size and red blood cell size in vertebrates. Blood Cells Mol. Dis., 27: 830-843.
18. Harrison, K.L., 1979. Fetal erythrocyte lifespan. J. Paediatr. Child Health, 15: 96-97.