Justifying the use of the bluish liquid from the African giant snail (*Achatina marginata*) in traditional male circumcision surgery in Western Nigeria

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In contemporary African tradition, the male child is circumcised. One of the requirements for successfully carrying out this surgical feat by the traditional birth attendants in the western part of Nigeria is to immediately bath the exposed penis surface with the fresh bluish liquid from the African giant snail (*Achatina marginata*). This study screened the bluish liquid for anti-bacteria/anti-fungi activities and examined it for coagulatory effect. The coagulatory effect was assessed through the Prothrombin time (PT) of three categories of people, (a) normal persons, (b) patients on warfarin, (c) hemophilic patients, and compared with the PT of calcified tissue thromboplastin (reference) on the same people. The anti-bacterial/anti-fungal effects were studied using three bacteria and three fungi grown on nutrient agar; the inhibitory effect of the bluish liquid on their growth was compared with that of standard antibacterial (gentamicin) and antifungal ( clotrimazole) drugs. The studies were carried out using the two known varieties of *A. marginata* (suturalis and ovum) in order to establish any variation in their effectiveness. Anti-bacterial or anti-fungal property was not exhibited by the bluish liquid, but a stronger (than the reference) coagulatory effect which was also effective on hemophilic blood was revealed in the bluish liquid from the two snail varieties. *A. marginata* ovum showed higher potency over the suturalis variety. Elemental analysis of the bluish liquid from the two snail varieties carried out showed three elements (calcium, magnesium and zinc) in relatively large amount when compared with other detected elements. The result on the prothrombin time justified the use of the snail bluish liquid as a strong blood coagulant while the elemental results supported the observed higher potency of the ovum snail variety over the suturalis variety. The result also suggests that the hemophiliacs can benefit from this liquid.

Key words: Male circumcision, Western Nigeria, blood clotting, *Achatina marginata* (African snail), prothrombin time, hemophiliacs.

INTRODUCTION

Male circumcision originally was notably and mostly known to be a religious ritual practiced among the Jews but has widely spread in conception and spanned over three millennia (Rubin, 2003). Circumcision as defined by the University of Michigan Health system in 2007 is the surgical removal of some or the entire foreskin (prepuce) from the penis. Religious male circumcision is considered a commandment from God in Judaism as recorded in the Holy Bible, and it is customary in many Christian churches in Africa; most Muslims also practice and see it as sunnah (Rizvi et al., 1999) hence it is of general

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acceptance, especially in the Middle East, North and West Africa (Weiss et al., 2007).

Medically, many reports have advocated it as a good preventive measure for a number of diseases like urinarytract infection, sexually transmitted diseases, penile cancer, prostate cancer, cervical cancer and acquired immunodeficiency syndrome (AIDS) (Weiss et al., 2007; Nigerian Punch, 2011). Throughout the world, human immunodeficiency virus (HIV) prevalence is reported to be generally lower in populations that practice male circumcision than in populations where most men are uncircumcised. Many trials have been reported that brought out the conclusion that male circumcision is an effective risk-reduction measure for men, and should be used in addition to other known strategies for the prevention of heterosexually acquired HIV infection in men (Joint UN programme on HIV/AIDS, 2008).

Traditionally in Western Nigeria, circumcision is usually performed at a very tender age (usually 8th or 10th day after birth) and very early in the morning by the local traditional birth attendant (TBA) who is usually a female. The bluish liquid from the giant African snail Achatina marginata is one of the materials required for this feat. Immediately the foreskin is surgically removed, the shell of the life snail is washed, broken at the tip end and the clear blue liquid which exudes is allowed to flow directly on the cut surface. No further dressing or medication is applied on this day; but on the second day either methylene blue dye or a preparation of some herbs in palm oil is applied on the wound with the aid of a feather; this is done daily after the child’s bath. The sore heals within one to two weeks. The question to address is, what is the role being played by the snail bluish liquid in this surgery, is it coagulatory or/and anti-bacterial/or anti-fungal?

Snails are the largest group of molluscs which constitutes the largest animal group after arthropods (Glick, 2005). Land snail’s habitats in Nigeria range from the dense tropical high forest in the south to the fringing riparian forests of the derived Guinea Savanna in the middle belt of the country (Yoloye, 1984). The snail meat have been established as a good source of protein (Ajayi et al., 1980) and they are relished delicacies among the Calabars, Itsekiris, Yorubas and many other coastal tribes of West Africa (Ajayi et al., 1980; Brender, 1992) who now breed them commercially to meet demand (Imevbore and Ademosun, 1988; Odaibo, 1997).

The giant African snail A. marginata exists in two different varieties, ovum and suturalis (Ajayi, 1980); both varieties were used in these studies in order to determine which of the two gives the better result; though the ovum variety is more commonly encountered and used (Odaibo, 1997; Ademolu et al., 2004).

Coagulation is a complex process by which blood forms clots to stop bleeding and begin repair of any damaged vessel. It is an important part of hemostasis wherein a damaged blood vessel wall is covered by a platelet and fibrin-containing clot (Dahl, 2000). Coagulation involves a cellular (platelet) and a protein (coagulation factor) component. It begins almost instantly after an injury to the blood vessel has damaged the endothelium; exposure of the blood to proteins such as tissue factor initiates changes to blood platelets which immediately form a plug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously.

Proteins in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands which strengthen the platelet plug. One of the tests commonly employed in the determination of blood clot is the prothrombin time (Dacie and Lewis, 2001).

Prothrombin time (PT) is a blood test that measures how long it takes a blood to clot and it is an important coagulatory test because it measures the presence and activity of five different blood clotting factors (Factors I, II, V, VII and X). It is a test of extrinsic and common pathway, and the normal PT is about 12 seconds (Fritsma, 2002).

These studies were designed to determine the effect of the snail liquid on some blood plasma samples clotting time and compare with that of a standard coagulator (thromboplastin). Likewise, the bacterial and fungal inhibitory effect of the blue liquid was studied using three bacteria and three fungi and compared with the inhibitory effect of standard antibacterial (gentamicin) and antifungal (clotrimazole) drugs. The studies further investigated the elemental composition of the blue liquid from the two snail varieties in order to explain variation in the observed clotting time, as some elements, calcium in particular, are essential for the clotting process. The atomic absorption spectrometer which is commonly used for elemental analysis of metals in solution (Ademolu et al., 2004; Fagburo et al., 2006; Adepoju-Bello et al., 2009; Momodu and Anyakora, 2010) was used for the analysis of the metal ions after proper digestion with aqua regia to get rid of organic impurities. A similar work carried out on snail reported solely on the elemental composition of the meat of four species of snail which included the two under study (Ademolu et al., 2004; Fagburo et al., 2006) but report on this bluish liquid was lacking.

MATERIALS AND METHODS

Materials used in the studies include ten snails, A. marginata (five of ovum and five of suturalis varieties); spectrophotometer (Perkin Elmer Analyst 200) acetylene gas, calcified tissue thromboplastin (Fisher Diagnostic Ltd, U.K), calcium chloride (May & Baker PLC), saboraund dextrose agar (SDA), Mueller Hilton agar (MHA) (Biotech, U.K), gentamicin, clotrimazole (Drugfield), Aqua regia (concentrated nitric and hydrochloric acids 3:1), sulphate salts of the elements under evaluation (Zn, Cu, Fe, Ca, Pb) (May & Baker PLC). Plasma from appropriate patients (three patients on warfarin and one hemophilic) were obtained from Hematology Outpatient Clinic of Lagos University Teaching Hospital. Normal (control) plasma was obtained from a healthy laboratory staff.
EXPERIMENTS

Antibacterial/antifungal screening

Three universal bottles each containing 20 ml Mueller Hilton agar were autoclaved at 121°C for 15 min and inoculated with 1 ml standardized suspension of Escherichia coli, Staphylococcus aureus and Bacillus subtilis, respectively after cooling to 40°C. Three bottles of Saboraund dextrose agar were similarly prepared and inoculated with 1 ml standard suspension of Aspergillus niger, Candidas albican and Trichophyton rubrum, respectively. Each of the inoculated agar was poured into three petri dishes, (two for test liquid that is, the bluish liquid from A. marginata ovum and A. marginata suturalis and the other for the control that is, the standard gentamicin or clotrimazole), allowed to set and four equal sized wells were bored with a Durham tube in each. Four different concentrations of the snail bluish liquid (100, 50, 25 and 12.5%) from the two snail varieties were filled into the wells of the two petri-dishes, respectively while four different concentrations (180, 80, 40 and 20) of the control (gentamicin) were filled into the wells of the other petri-dish of each bacterium containing agar with corresponding labels for identification as shown in the table of results. Incubation was done at 37°C for 24 h, after which the plates were inspected for zones of inhibition. The same procedures were carried out for the anti-fungi screening using 800, 400, 2000 and 100 μg of clotrimazole as control and incubating at room temperature. preparation of blood plasma

Nine milliliters (9 ml) of blood obtained from the patient’s antecubital fossa of the arm through a clean puncture at the vein was delivered into a 15 ml tube containing one ml of 0.1 M trisodium citrate. The content of the tube was properly mixed and then centrifuged at 40,000 rpm for 15 min. The supernatant plasma was gently removed using a pasteur pipette and used immediately.

One-stage prothrombin time

A one stage prothrombin time test was carried out using calcified tissue thromboplastin on both test and control plasma. Another series of the test were carried out where the snail bluish water was substituted for the calcified tissue thromboplastin. Exactly 0.1 ml of fresh normal or test plasma was delivered into the bottom of a 75 x 10 mm glass test-tubes placed in a water-bath at 37°C and equal volume of thromboplastin was added. A stop watch was started immediately and the time taken for the plasma to clot was recorded. Similarly, 0.1 ml of fresh plasma (obtained from three patients on warfarin and one hemophilic patient) were delivered into 75 x 10 mm glass test-tubes placed in water-bath at 37°C and tested using 0.1 ml of the freshly obtained bluish liquid from the two varieties of snail and tissue thromboplastin. The stop watch was immediately started to record the time taken for clot to appear. Each procedure was carried out in duplicate and the average time recorded.

Elemental composition

Each sample (5 ml) of the two snails’ water was digested separately in equal volume of aqua regia in order to get rid of all organic impurities and was then prepared into standard solutions using distilled de-ionized water. Standard solutions of each elemental salt in three different concentrations (2, 4 and 6 ppm) were prepared and their various absorbances were determined using the atomic absorption spectrometer (Perkin Elmer Analyst 200) in order to plot a calibration curve for each element to be quantified. The absorbance of the prepared standard snail waters was also taken for each element, absorbance readings were taken in triplicates in order to get an average value. The concentration of each element was then obtained from the absorbance regression on each calibration curve.

RESULTS AND DISCUSSION

The bluish water from the two snail varieties lacks any anti-microbial/anti-fungi activity as no inhibition was observed even in the growth of media containing the highest concentration (100%) of the snail’s liquid (Table 1). Prothrombin result shows prominent clotting ability which is more potent than that of calcified thromboplastin that was used as reference. The time taken by the snail’s water to clot serum from all the tested patients was shorter than the time taken by the calcified thromboplastin, with the liquid of the ovum variety showing higher potency. While the control could not clot hemophilic blood after 10 min, the bluish water from both varieties of the snail was able to do this before the expiration of 10 min, with ovum and suturalis taking 6.4 and 7.9 minutes, respectively.

Apart from the fact that the ovum variety is more commonly distributed in Nigeria, this result supports its choice as the preferred variety at the circumcision table. The elemental analysis result also corroborate this; for all the elements analyzed with the exception of manganese, the liquid from the ovum variety recorded higher concentrations which in some cases (Magnesium and Calcium) actually doubled the value obtained for the suturalis variety. This finding differs from the report of Fagbuaor et al. (2006) on the meat in which only small variations existed in the mineral content of the two varieties. The extrinsic mechanism that initiates the formation of prothrombin activator begins with the traumatized vascular wall or extra vascular tissues and progresses stepwise in the presence of calcium as shown (Dacie and Lewis, 2001).

Firstly, the traumatized tissue releases a complex of several factors called tissue factor or tissue thromboplastin which is composed of phospholipids from the tissue membranes and a lipoprotein complex which contain an important proteolytic enzyme.

Secondly, the lipoprotein complex of the tissue factor complexes with blood coagulation Factor VII and in the presence of calcium ions, acts enzymatically on Factor X to form activated Factor X designated as Factor Xa. Factor Xa combines immediately with Factor V as well as the phospholipids released from the platelets to form the complex called prothrombin activator and within few seconds in the presence of calcium ion, this splits prothrombin to form thrombin. Thrombin is a proteolytic enzyme which acts on fibrinogen to form the fibrin monomers which polymerize each other to form the long fibrin fibers that form the reticulum of the clot.

The essential role of calcium in the processes of blood clotting has long been established (Dacie and Lewis,
Table 1. Anti-microbial/anti-fungi: Zone of Inhibition (mm) at stated concentrations.

| Drug (µg) | Test organisms | Drug (µg) | Test organisms |
|-----------|----------------|-----------|----------------|
|           | Gentamicin     | Clotrimazole | A. niger   |
|           | B. subtilis    | E. coli    | C. albicans |
| 20        | 19.0           | 20.0       | 14.0        |
| 40        | 20.0           | 21.5       | 15.0        |
| 80        | 24.0           | 22.5       | 17.0        |
| 160       | 26.0           | 25.5       | 20.0        |

| Drug (µg) | Test organisms | Drug (µg) | Test organisms |
|-----------|----------------|-----------|----------------|
|           | Clotrimazole   | A. niger  |
|           | S. aureus      | C. albicans |
| 200       | 15.0           | 20.0       |
| 400       | 17.0           | 24.0       |
| 800       | 20.0           | 28.0       |

A. marginata (suturalis) %

| %  | Activity |
|----|----------|
| 12.5 | No activity |
| 25   | 25 |
| 50   | 50 |
| 100  | 100 |

A. marginata (ovum) %

| %  | Activity |
|----|----------|
| 12.5 | No activity |
| 25   | 25 |
| 50   | 50 |
| 100  | 100 |

2001; Heneghan et al., 2006), and that the bluish liquid of the African giant snail contains it in high amount can explain why its application immediately on the exposed penis after the foreskin is removed during circumcision is imperative. It facilitates clotting and prevents excessive bleeding. The application of methylene blue or/and other oil dispersed herbs from the second day of the operation on the other hand, suggests awareness that the bluish liquid from the snail cannot prevent microbial infestation; hence these were probably used to act as anti-bacterial agents. However, already studied plants with antibacteria activity like Cymbopogon flexuosus (lemongrass) and C. nardus (citronella) (Innsan et al., 2011) could be used in place of the methylene blue at least to avoid the mess of its stain.

Apart from calcium, magnesium and zinc elements were also found in high amount (Table 3) in the bluish liquid from the snail which clotted the hemophilic blood that the calcified thromboplastin (control) could not do (Table 2). Magnesium and zinc are cofactors in the synthesis of some proteins (Rutin, 1975; Narayan et al., 1997), and bearing in mind that the various clotting factors are proteineous, one may then reason that these other two elements may have an additional role to play alongside calcium in the clotting of blood, particularly in the hemophiliacs. In countries such as East and South Africa where circumcision is being advocated for AIDS control and performed at puberty, report of excessive bleeding has been given as a major setback (Nigerian Punch, 2011); use of this bluish liquid from the African giant snail (the ovum variety) may provide a succor.

Conclusion

The result on the prothrombin time justified the use of the snail bluish liquid as a strong blood coagulant, useful in preventing excessive blood loss during and after surgery while the elemental results supported the observed higher potency of the ovum snail variety over the suturalis variety. It further suggests that the hemophiliacs can benefit from this liquid. Further studies on this snail liquid...
Table 2. Prothrombin time.

| Sample                  | Average clotting time (seconds) ± standard deviation |
|-------------------------|-----------------------------------------------------|
| Blood plasma sample     | Thromboplastin (reference) Bluish liquid of *A. marginata* (suturalis) Bluish liquid of *A. marginata* (ovum) |
| Normal (control)        | 11.6±0.3     8.5±2.1                8.0±1.4                |
| Patient 1 (on warfarin) | 34.4±0.2     16.0±1.4                13.5±2.1                |
| Patient 2 (on warfarin) | 37.5±0.3     17.2±1.4                15.5±2.1                |
| Patient 3 (on warfarin) | 23.0±0.1     15.5±0.7                13.0±0.2                |
| Hemophiliac patient     | No clotting after 10 min 415.0±4.7 (7.9 min) 385.0±2.8 (6.4 min) |

Table 3. Elemental composition.

| Element         | *A. marginata* (suturalis) Mean concentration (mg/L) ± standard deviation | *A. marginata* (ovum) Mean concentration (mg/L) ± standard deviation |
|-----------------|--------------------------------------------------------------------------|--------------------------------------------------------------------------|
| Lead (Pb)       | 1.1176±0.0021                                                            | 1.5294±0.0032                                                            |
| Iron (Fe)       | 2.3989±0.0054                                                            | 2.5500±0.0108                                                            |
| Copper (Cu)     | 0.5870±0.0117                                                            | 0.6114±0.0024                                                            |
| Magnesium (Mg)  | 25.736±0.0328                                                             | 54.9717±3.3190                                                            |
| Manganese (Mn)  | 4.2541±0.0361                                                             | 2.7403±0.0052                                                            |
| Calcium (Ca)    | 22.450±0.073                                                             | 47.2487±2.6510                                                            |
| Potassium (K)   | 4.3214±0.0106                                                             | 7.7381±0.0946                                                            |
| Zinc (Zn)       | 26.326±0.463                                                             | 35.0233±1.7940                                                            |

are recommended in order to establish the factor(s) inherent in it that support the clotting of hemophiliac blood.

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REFERENCES

Ademolu KO, Idowu AB, Mafiana CF, Osinowo OA (2004). Performance, Proximate and Mineral Analysis of African giant land snail (*Achatina marginata*) fed on different Nitrogen Sources. Afr. J. Biotech. 3(8):412-417.

Adepoju-Bello AA, Ojimolade OO, Ayoola GA, Coker HAB (2009). Quantitative Analysis of Some Toxic Metals in Domestic Water Obtained from Lagos Metropolis. Nig. J. Pharm. 42:57-60.

Ajayi SS, Tewe SO, Milligan JK. (1980). Influence of Season on Aestivation and Behavior of the Forest African Giant Land Snail, *Achatina marginata*. Bull. Annual Health Proc. 28:328.

Ajayi SS. (1980). Observation on the biology and nutritive value of the African giant snail (*Achatina marginata*). East Afr. Wild J. 16:85-95.

Brender A (1992). Meat and Meat Products in Human Nutrition in Developing Countries. FAO Food and Nutrition. Rome. p. 53.

Dacie JV, Lewis SM (2001). Practical Hematology, Ninth Edition.

Dahl B (2000). Blood coagulation. Lancet 355:1627-1632.

Fagburo O, Oso JA, Edward JB, Ogunleye RF (2006). Nutritional status of four species of giant snails in Nigeria. J. Zhejiang Univ. Sci. B. 7(9):686-689.
Fritsma GA (2002). Evaluation of Hemostasis. ‘Hematology: Clinical Principles and Applications.” In: Rodak BF, Fritsma GA, Doig K (Eds.), Hematology: Clinical Principles and Applications. WB. Saunders Company, Philadelphia. pp. 719-53.

Glick LB (2005). Marked in your flesh: circumcision from ancient Judea to modern America. New York Oxford University Press.

Heneghan C, Alonso-Coello P, Garcia-Alamino JM, Perera R, Meats E, Glasziou P (2006). Self-monitoring of oral anticoagulation: a systematic review and meta-analysis. Lancet 367(9508):404-411.

Imevbore EA, Ademosun AA (1988). The nutritive value of the African giant snail, “Achatina marginata”. J. Anim. Prod. Res. 8(2):76-87

Innsan MF, Shahril MH, Samihah MS, Asma OS, Radzi SM, Abd Jalil AK, Hanina MN (2011). Pharmacodynamic properties of essential oils from Cymbopogon species. Afr. J. Pharm. Pharmacol. 5(24):2676-2679.

Joint UN programme on HIV/AIDS (2008). Safe, voluntary, informed male circumcision and comprehensive HIV prevention programming; Guidance for decision makers on human rights, ethical and legal considerations. UNAIDS/08.19E/JC1552E.

Momodu MA, Anyakora CA (2010). Heavy Metal Contamination of Ground Water: The Surulere Case Study. Res. J. Environ. Earth Sci. 2(1):39-43.

Narayan VA, Kariwaccki RW, Caradonna JP (1997). Structure of Zinc Finger Domains from Transcript Factor SP1; Insights into sequence-specific protein-DNA recognition. J. Biol. Chem. 272:7801-7809.

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Rubin N (2003). Brit. Milah: a study of change in custom. In: Wyner ME (ed.), The covenant of circumcision: new perspectives on an ancient Jewish rite. Brandeis University Press, Lebanon NH. pp. 87-97, 223-228.

Rutin H (1975). Central role for Magnesium in co-ordinate control of metabolism and growth in animal cells. Proceedings of the National Academy of Science of the USA. 72(9):3551-3555.

The Holy Bible (RSV). Genesis 17:9–14.

University of Michigan Health system (2007). Pain and your Infant: Medical Procedures, Circumcision and Teething.

Weiss H, Polonsky J, Bailey R, Hankins C, Halperin D, Schmid G (2007). Male Circumcision: global trends and determinants of prevalence, safety and acceptability. WHO and Joint UN Programme on HIV/AIDS 2007. WHO Press, Geneva, Switzerland.

Yoloye VL (1984). Molluscs for Mankind. Inaugural Lecture, University of Ilorin, Ilorin, Nigeria.

Nigerian Punch (2011). Slow progress in circumcising men to fight AIDS in Africa, September 29, 2011 (New York Times) p.46.

Odaibo AB (1997). Snail and Snail Farming; Nigeria Edible Land Snail, Vol. 1. Stirling-Harden Publishers, Ibadan. pp. 1-11.

Rizvi SAH, Naqvi SA, Hussain M, Hasan AS (1999). “Religious circumcision: a Muslim view”. BJU Int. 83:13–16.