Effects of the bluish liquid (hemolymph) from the African giant snail (*Achatina marginata*) on the blood coagulation time and erythropoietic volume

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The bluish liquid (hemolymph) from the African giant snail designated snail bluish liquid (SBL) was investigated for its coagulatory potency *in vitro* using human blood from different patients and *in vivo* using the Wistar albino rat. Its effect on the erythrocyte volume (PCV) during the period of administration in the rat was also studied. Coagulatory potency was studied in terms of the prothrombin time (PT) by comparing the PT of the blood obtained from the patients when mixed with the SBL with that obtained when mixed with a standard coagulatory agent. The *in vivo* study administered the SBL at different doses to groups of matured Wistar rats for a period of time, determined the PT of their withdrawn blood and compared with that of a control group that did not receive SBL. At the same time the collected blood was analyzed for its red blood volume (PCV) using the hematocrit reader. All obtained data were statistically analyzed. Results showed a significant shorter PT with SBL when compared with the calcified tissue thromboplastin standard used in the *in vitro* study, and the *in vivo* result also showed dose dependent significant reduction in clotting time and increase in PCV after the SBL administration. However, the increase recorded in PCV showed a non significant increase at the low doses of 50 and 100 ml/kg; the higher doses that showed significant increases also exhibited some lethality. In conclusion, SBL is seen as a good first aid agent to arrest external bleeding. Its oral administration at low doses (<200 ml/kg) may boost blood formation but may also precipitate blood clot while the higher doses must be avoided because of toxicity.

Key words: Snail, coagulatory agent, thromboplastin, blood, hemophiliac.

INTRODUCTION

The African giant snail (*Achatina marginata*) is a land, nocturnal, invertebrate animal of the Phylum Mollusca (Ademolu et al., 2006). It is found mostly under stones or litter of decaying organic matter during the day time. In West Africa, its favorable habitat for survival is the dense high forest and the fringe of the derived Guinea Savannh (Dede et al., 2003; Ademolu et al., 2006). Apart from reported high nutritional value (Agbogidi et al., 2008; Ogogo et al., 2011) which has made it economically important in the region, it is also used in traditional...
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 coagulatary 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 for man is about 12 s (Fritsma, 2002). While the PCV gives a measure of the amount of blood cells.

These studies were designed to determine: (a) the coagulatory effects of SBL on (i) the clotting time of the blood plasma samples obtained from three different individuals (normal person, patients using warfarin and hemophilic patient) when mixed (SBL) in vitro and (ii) the clotting time of the obtained plasma after it (SBL) has been administered in different doses in vivo to rat via oral feeding for two weeks. The in vivo study had a control group that served as the reference with which observed results were compared; (b) the effect the bluish liquid has on the blood cell formation by measuring the packed cells volume (PVC) of the rat’s blood after the two-week oral administration of the SBL.

**MATERIALS AND METHODS**

Sixty snails (A. marginata) variety ovum were purchased from snail sellers in Oyingbo market in Lagos State. They were identified by Professor R. I. Egbonmwam of the Department of Zoology, University of Lagos. 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.

**In vitro study**

**Preparation of blood plasma**

Nine milliliters of blood obtained from the patient’s antecubital fossa of the arm through a clean puncture at the vein wall was delivered into a 15 ml tube containing 1 ml of 0.1 M trisodium citrate. The content of the tube was properly mixed and then centrifuged at 4,000 rpm for 15 min. With the aid of a Pasteur pipette the supernatant plasma was gently removed and used immediately.

**One-stage prothrombin time**

A one stage prothrombin time test was carried out using calcified tissue thromboplastin (CTP) on both test and control plasma. Another series of the test were similarly carried out where the SBL was substituted for the CTP. Exactly 0.1 ml of either fresh normal or test plasma (obtained from three patients using warfarin and one hemophilic patient) was delivered into the bottom of a 75 × 10 mm glass test-tubes placed in a water-bath at 37°C and equal volume of CTP was added. A stop watch was started immediately and the time taken for the plasma to clot was recorded. This same procedure was carried out again using 0.1 ml of the freshly obtained SBL in place of CTP. Each procedure was carried out in duplicate and the average time recorded (Table 1).

**In vivo study**

Twenty five adult male albino Wistar rats obtained from the animal house of College of Medicine, University of Lagos, Ili-araba Campus were allowed to acclimatize for one week and divided into five groups of 5 rats per group. The first group was orally administered 200 ml/kg of distilled water to serve as control. The remaining four groups were respectively administered with 50, 100, 200 and 300 ml/kg of the SBL orally. The SBL was collected in sterile bottle every morning and administered immediately to the rats for 14 consecutive days. After this blood was withdrawn via ocular vein puncture from the rats and plasma was prepared from
Table 1. *In vitro* study result: prothrombin time (PT) of three differently sourced blood plasma after being mixed with SBL.

| Source of plasma | Normal | Patients using warfarin 5 mg/day | Haemophiliac |
|------------------|--------|----------------------------------|--------------|
| Patient identification | A | B | C | H |
| Mean time with CTP (Reference) (s) | 11.6 ± 0.3 | 34.4 ± 0.2 | 37.5 ± 0.3 | 33.0 ± 0.1 | 30.0 ± 1.1 |
| Mean time with SBL (s) | 8.0 ± 1.4 | 13.5 ± 2.1 | 15.5 ± 2.1 | 13.0 ± 0.2 | 235 ± 28.0 |
| Mean reduction in time (s) | 3.6 | 20.9 | 22.0 | 20.0 | - |
| Reduction time (%) | 31.0* | 60.8** | 58.0** | 60.6** | - |

Table 2. *In vivo* study result: Prothrombin time (s) of rats administered with different doses of SBL.

| Group | Dose (mg/kg) | Rat 1 | Rat 2 | Rat 3 | Rat 4 | Rat 5 | Mean ± SEM | Mean reduction in time |
|-------|--------------|-------|-------|-------|-------|-------|------------|-----------------------|
| Control | 200 (H₂O) | 56    | 49    | 55    | 48    | 50    | 51.6 ± 3.26 | Not applicable (Reference) |
| Test | 50 (SBL) | 51    | 43    | 50    | 42    | 44    | 46.0 ± 3.74 | 5.6 (12.2%) |
| Test | 100 (SBL) | 28    | 23    | 27    | 21    | 32    | 26.2 ± 3.87** | 24.4 (49.2%) |
| Test | 200 (SBL) | 25    | 22    | 25    | 20    | Died | 23.0 ± 2.12** | 28.2 (55.4%) |
| Test | 300 (SBL) | Died | Died | Died | 23    | Died | 22.0 ± 1.00** | 29.6 (57.4%) |

Statistical evaluation (t-test) of the clotting time at 99% confidence (p = 0.01) limit for the studies indicate that the results obtained from the control (using CTP) were significantly different from the results obtained for the SBL. Values are expressed as mean ± standard error of mean. *Indicates a significant difference (p<0.05) and **indicates a highly significant difference (p<0.01).

Table 3. Percent PCV of rats dosed with different doses of SBL.

| Group | Dose (ml/kg) | Rat 1 | Rat 2 | Rat 3 | Rat 4 | Rat 5 | Mean ± SEM |
|-------|--------------|-------|-------|-------|-------|-------|------------|
| Control | 200 | 32    | 35    | 38    | 32    | 36    | 34.6 ± 2.33 |
| Test | 50 | 32    | 36    | 38    | 33    | 37    | 35.2 ± 2.09 |
| Test | 100 | 34    | 38    | 40    | 36    | 38    | 37.2 ± 2.04 |
| Test | 200 | 37    | 41    | 42    | 38    | 41    | 39.8 ± 1.94* |
| Test | 300 | Died | Died | 45    | Died | Died | 43.5 ± 1.5* |

*Indicates a significant difference (p<0.05) at 99% confident level when compared with the control.

the collected blood samples as done in the *in vitro* test; the PT test was performed in duplicate for each animal's plasma as follows: For each test, two tubes were arranged in the water bath. To each tube, 100 µl of the plasma was added and incubated at 37°C for 3 min. 200 µl of CTP was added to each sample at a time, immediately after which a stopwatch was started. The prothrombin end point for each, that is, the time at which visible clot was observed was noted and the average value was recorded (Table 2).

**Packed cell volume (PCV) determination**

The blood collected via ocular vein puncture for each dosed animal was transferred into pre-labeled EDTA bottles and gently mixed by inversion. Three-quarter full capillary blood sample was taken from each EDTA bottle and the capillary top was sealed with plastacin. The tubes were then arranged in the haematocrit centrifuge with the sealed portion facing the outward part of the centrifuge. The samples were centrifuged at 10,000 rpm or 5 min. The micro-haematocrit reader was used to read the PCV value after the centrifugation (Table 3).

**RESULTS AND DISCUSSION**

Both the *in vitro* and *in vivo* results show significant reduction in the observed clotting time after the introduction of the SBL. This fact is of tremendous importance medically when the snail water is taken internally at doses higher than 50 ml/kg as it signals potential danger towards thrombosis which is majorly responsible for precipitating stroke, a condition which is becoming prevalent in Nigeria. The general consumption of snail however is limited to its meat which has been confirmed as nutritious and rich in protein and seldom is this liquid taken internally. On the other hand internal administration of this bluish water from the snail may augur well for the hemophiliacs to who blood clotting is a major problem as shown in Table 1 result. This potential effect to reduce the time for blood both *in vitro* and *in vivo* to clot may be the reason for its use to reduce blood loss during labour (delivery) in women (Agbogidi et al., 2008). The external use of the liquid which shorten the clotting time significantly (Table 1), is well desired in the cases of injuries that leave the blood vessels open like a cut, accident wounds, situations that demand quick arrest of blood flow and this justifies its use at the traditional circumcision surgery table to arrest bleeding. This
property makes snail very relevant as a first aid in arresting bleeding in any rural area (where a modern health facility is not available) when there is an injury to the blood vessel. The pro clotting property of the bluish liquid is enhanced by its high calcium content which is one of the major requirements for the process of blood clotting.

The packed cell volume (PCV) recorded an insignificant dose dependent increase in value and showed lethality at 300 ml/kg (as three out of the five rats died in the course of the study) and this is the dose at which a significant increase was recorded. This positive effect on PCV value is expected for it has been shown by Olagbende-Dada et al. (2013) that the SBL contains both iron (Fe) and copper (Cu) elements that are required for blood cell formation. The observed increase in PCV suggests that the SBL could be of use (within its safety margin) in preventing or treating mild anemia; however, its use is not advisable because of its tendency to form blood clot.

Conclusion

SBL can be used as a good first aid agent to arrest external bleeding of damaged blood vessels. Its oral administration at low doses (<200 ml/kg) may boost blood formation but may also precipitate blood clot while the higher doses must be avoided because of toxicity.

Conflict of interest

Authors declare that there are no conflicts of interest.

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