BIOLOGICAL ACTIVITY OF BARTOGENIC ACID GLUCOSYL HEXAACETATE ISOLATED FROM COMBRETUM MICRANTHUM ON PLASMA RECALCIFICATION TIME OF NAJA KATIENSIS AND BITIS ARIETANS VENOMS

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ABSTRACT

Biological effect of bartogenic acid 28-\(\beta\)-D-glucosyl hexaacetate (BA), a triterpenoid, isolated from the root of Combretum micranthum on recalcification time of bovine plasma treated with Naja katiensis and Bitis arietans venoms was investigated. The plasma recalcification time (PRT) was found to be effectively reduced in a dose dependent fashion. Inhibition of 73.2 % at 1 mg/ml BA on the elongation of the PRT by N. katiensis venom was obtained. This was however found to decrease to a low level of 5.4 % when 0.001 mg/ml of BA was used. Similarly, the highest and lowest inhibition values of 47.7 % and 28.6 % at 1 mg/ml and 0.001 mg/ml doses of BA on the elongation of the PRT by B. arietans venom were obtained respectively. This study showed that BA could effectively serve as an inhibitor of snake venom induced bleeding, as it was shown to quantitatively reduce the plasma recalcification time.

KEYWORDS: Triterpenoid, plasma, inhibitor, venom, recalcification time.
INTRODUCTION
Venom is a secretion, produced in a specialized gland in an animal. It contains molecules capable of disrupting normal physiological or biochemical processes when delivered to a target animal through the infliction of wound so as to facilitate feeding or defense by the venom producing animal.\(^1\)\(^-\)\(^2\) Venoms typically comprise a mixture of protein and peptides (commonly referred to as toxins), salts and organic components, such as amino acids and neurotransmitters.\(^1\)\(^-\)\(^4\) Venoms are sub-divided into cytotoxins, cardiotoxins, neurotoxins, and hemotoxins.\(^5\)

*Bitis arietans* is a species of viper which occupies densely populated habitats throughout the Middle East and savannah areas of sub-Saharan Africa.\(^6\) The precise number of human fatalities due to *B. arietans* envenoming is unknown. However, bites by this species are widely believed to contribute to a substantial proportion of the estimated 43,000 deaths from snake bite reported in Africa annually.\(^7\) *B. arietans* venom is considered the most toxic of any viper species with a murine LD\(_{50}\) 9–13 µg/mouse.\(^8\) *Naja katiensis*, otherwise called Mali cobra or Katian spitting cobra is one of the *Naja* species. They are a medically important group of snakes due to the number of bites and fatalities they cause across their geographical range. They range throughout Africa and belong to the *Elapidae* family.\(^9\)

Snakebite envenoming constitutes a highly relevant public health issue on a global basis, although overlooked by health authorities in many parts of the world.\(^10\)\(^-\)\(^13\) The actual incidence of snakebite envenoming worldwide and its associated mortality are difficult to estimate, since there are many countries where this pathology is not appropriately reported. Adequate treatment of snakebite envenoming is critically dependent on the ability of antivenoms to reverse venom-induced coagulopathy, hemorrhage, hypotensive shock and other signs of systemic envenoming.

Plants are known to contain secondary metabolites like alkaloids\(^14\)\(^-\)\(^15\), phenolics\(^16\)\(^-\)\(^18\), steroids\(^19\) and terpenoids\(^20\)\(^-\)\(^22\)\(^-\)\(^15\) that have antivenin activities. These components present in plants, makes plants serve as alternatives for treatment of poisonous snake bites.\(^23\)\(^-\)\(^24\) Terpenes and their glucosides have been widely investigated in the search for new alternative pharmaceutical active compounds. Pentacyclic triterpenes are often associated with antivenom activity while no activity was reported for a tetracyclic triterpene. Thus, the five-ring structure of triterpene has been suggested to be an essential component for the antivenom and anti-inflammatory activity for an active pharmacophore.\(^11\) Here, we report on the effect
of bartogenic acid 28-β-D-glucosyl hexaacetate, a pentacyclic triterpenoid glucoside isolated from the root of *Combretum micranthum* on recalcification time of bovine plasma treated with *Naja katiensis* and *Bitis arietans* venoms.

**MATERIALS AND METHODS**

**Venom collection**

*Naja katiensis* and *Bitis arietans* venoms were a gift from Prof. M. S. Abubakar of the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. Stock solutions of 10 mg/ml of the venoms in a phosphate buffer saline (PBS) were prepared separately when needed for use.

**Reagents**

General laboratory and inorganic chemicals of analytical grade were obtained from Sigma (St. Louis, USA).

**Sample Preparation**

The purified BA sample was obtained from research partners in the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria-Nigeria. A stock solution of 1 mg/ml BA was prepared in PBS, pH 7.4. Dilutions of 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of the BA were made from the stock respectively.

Fresh bovine red blood cells were collected in heparinized tubes and then centrifuged to obtain the plasma.

**Determination of Effect of Bartogenic Acid Glucosyl Hexaacetate on Plasma Recalcification Time**

The method as described by Theakson and Reid [25] was used to determine the effect of BA on plasma recalcification time in incubated plasma (with *Naja katiensis* or *Bitis arietans*) and non-incubated plasma. A set of 11 tubes containing 100 µl of heparinized bovine plasma were incubated in a water bath at 37 °C. To four of the tubes above, 100 µl of 1.0, 0.1, 0.01 and 0.001 mg/ml dilutions of BA were added separately into each respectively. This was then followed with addition of 100 µl of PBS into the four tubes. To the resulting mixture, 25 mM CaCl₂ was added and the recalcification time recorded. To another set of four tubes containing 100 µl of the heparinized plasma, 100 µl of 10 mg/ml venom was added to each. This was followed with the addition of 100 µl of 1.0, 0.1, 0.01 and 0.001 mg/ml dilutions of
BA respectively. Then, 100 µl of 25 mM CaCl₂ was added into the four tubes and the recalcification time recorded. To the remaining set of three tubes containing heparinized plasma, 25 mM CaCl₂ (100 µl), 10 mg/ml venom (100 µl) and PBS (100 µl) each were added respectively, then 100 µl PBS was added into the three tubes. Finally, 100 µl of 25 mM CaCl₂ was added to each of the resulting mixture and the recalcification time recorded. These were conducted in triplicates and the recalcification time was given as mean ± SD.

RESULT

Figure 1: Effect of bartogenic acid glucosyl hexaacetate isolated from *C. micranthum* on plasma recalcification time of *N. katiensis* venom

Key: P = plasma; C = CaCl₂; PBS = phosphate buffer saline; V = venom (1 g/100ml); E = extract (1 mg/ml); X = dilution

Figure 2: Effect of bartogenic acid glucosyl hexaacetate isolated from *C. micranthum* on plasma recalcification time of *B. arientans* venom

Key: P = plasma; C = CaCl₂; PBS = phosphate buffer saline; V = venom (1 g/100ml); E = extract (1 mg/ml); X = dilution
The effect of BA on the recalcification time on plasma incubated with *Naja katiensis* venom is depicted in figure 1. Plasma incubated (with venom) only had a recalcification time of 16.01 ± 0.75 minutes. The recalcification times of non-incubated (normal) plasma treated with 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of BA were 2.56 ± 0.43, 2.97 ± 0.56, 3.7 ± 0.71 and 4.06 ± 0.63 respectively. While incubated plasma treated with 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of BA had recalcification times of 4.29 ± 0.45, 4.79 ± 0.51, 5.56 ± 0.63 and 15.15 ± 0.21 minutes respectively.

The effect of BA on the recalcification time on plasma incubated with *Bitis arietans* venom is depicted in figure 2. The recalcification time of plasma incubated (with venom) only was 18.83 ± 0.52 minutes. The recalcification times of non-incubated (normal) plasma treated with 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of BA were 4.21 ± 0.74, 5.38 ± 0.43, 6.21 ± 0.67 and 6.64 ± 0.34 respectively. While the recalcification times for incubated plasma treated with 1 mg/ml, 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of bartogenic acid glucosyl hexaacetate were 9.84 ± 0.73, 10.57 ± 0.58, 13.14 ± 0.26 and 13.44 ± 0.64 minutes respectively.

**DISCUSSION**

Snake venoms contain a variety of bioactive substances that influence homeostasis, thrombosis, and coagulation of mammalian blood.\(^{[26–29]}\) Many snake venom proteins have been isolated, that affect platelet plug formation by interacting either with platelet integrins, membrane glycoprotein Ib (GPIb), or plasma von Willebrand factor (VWF). Among them, disintegrins purified from various snake venoms are strong inhibitors of platelet aggregation.\(^{[30]}\)

*Naja naja* kaouthia venom has been found to contain a metalloproteinase (kouthiagin) with two disintegrin-like sequences.\(^{[31]}\) It is found to cleave a single site of VWF resulting in the disruption of the platelet agglutination-inducing activity of VWF.\(^{[32]}\)

This report presents the use of a glycosylated bartogenic acid; a β-amyrin containing pentacyclic triterpene in preventing the elongation of plasma recalcification/thrombin time instigated by *N. katiensis* and *B. arietans* venoms in vitro. The results obtained, clearly revealed a reversal of the increase in plasma recalcification time resulting from the effect of both venoms in a dose dependent manner by the presence of bartogenic acid 28-β-D-glucosyl hexaacetate. This compound therefore can be said to have found its way to inhibit the plasma
recalcification time by either interfering directly or indirectly with the mechanisms involved in blood clotting where different proteins, enzymes eg fibrinogenase are involved in the blood clot formation.

Although, no report on the effect of pentacyclic triterpene like bartogenic acid on snake envenomation has been made. This report therefore represent a novel finding with regards to the biological effect of the pentacyclic triterpenes on *N. katiensis* and *B. arietans* induced PRT elongation. The positive effect of BA on the plasma recalcification time could be attributed to its β-amyrin structure; reported to be having pharmacological properties.\[^{33-35}\]

This could be due to the ability of the β-amyrin to interfere with recalcification cascade in a positive manner earlier antagonized by these venoms like preventing the cleavage of a site in the VWF as instigated by *Naja* spp.

**CONCLUSION**

This study revealed that bartogenic acid 28-β-D-glucosyl hexaacetate, effectively halted the elongation of the plasma recalcification time by *Naja katiensis* and *Bitis arietans*. The findings could significantly help in the development of potent antivenin targeted towards ameliorating the biological effect of such venoms.

**REFERENCE**


