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EFFECT OF KIHADALACTONE ON PLASMA RECALCIFICATION TIME OF NAJA KATIENSIS AND BITIS ARIETANS VENOMS TREATED BOVINE PLASMA

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ABSTRACT

The effect of a limonoid (Kihadalactone) on recalcification time of bovine plasma treated with *Naja katiensis* and *Bitis arietans* venoms was investigated. The plasma recalcification time was found to be effectively reduced in a dose dependent pattern. In plasma incubated with *N. katiensis* venom, the highest inhibitory activity of 42.3 % at 1 mg/ml dose of kihadalactone was obtained, this was however found to decrease to a lower inhibition value of 27.3 % at 0.001 mg/ml dose. Similarly, in plasma incubated with *B. arietans* venom, an inhibitory activity of 51.2 % at 1 mg/ml dose of kihadalacetone and a low activity of 33.6 % inhibition of the increase at 0.001 mg/ml were obtained. This study showed that kihadalactone, could effectively serve as an antidote for the reversal of increase in plasma recalcification time posed by both snake venoms.

KEY WORDS: *Bitis arietans, Naja katiensis,* snake venom and plasma recalcification time.

INTRODUCTION

Snake venoms are complex mixtures of proteins, peptides, carbohydrates, lipids, metal ions and organic compounds. However, proteins and peptides accounts for about 90 % of their dry weight.[1] The proteins found in the venom are mainly enzymatic or non-enzymatic. About 20

types of toxic enzymes are found in snake venoms worldwide, and 12 of which are found in all types of snake venom.[2] Snake venoms have been shown to interfere with the blood coagulation factors and platelet aggregation leading persistent bleeding in victims.[2] They are also known to have diverse biological activities, one of which is fibrinolytic activity that fascilitates the enhancement of plasma recalcification time (PRT) or plasma thrombin time. Plasma recalcification time is basically a measure of time taken for plasma to recalcify or form clot. It is a physiological process that involves the interplay of calcium ions, clotting factors among other enzymes. Serum therapy is most wildly use in the treatment for snake bite, this efficiently neutralizes the systemic effect, thus preventing the death of victims. However, antivenoms have some disadvantages which include adverse reactions without neutralizing or reversing the local tissue damage[3] thus limiting their efficiency.

Bitis arietans also called (Puff adder) is a viper and highly toxic venomous snake that is responsible for a large proportion of the venomous snakebites in sub-Saharan Africa, where it is indigenous. Puff adder envenomation causes tissue necrosis, hypotension, coagulopathy, thrombocytopenia, and spontaneous bleeding.[4] *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.[4]

Snake venom antiserum development and standardization is expensive and requires ideal storage conditions.[5] These storage facilities are limiting in endemic areas of most developing countries. Plants are popular alternatives for the treatment of poisonous snake bites.[6,7] Quite a number of antivenin compounds have been isolated from some of these plants.[8,10]

Limonoids are phytochemicals abundant in citrus fruits and have been found to have several medicinal uses and potentials.[11] Plant preparations from different sources are currently in use for the treatment of snake bites but the scientific basis/validation for such practices is still lacking. The plant preparations on their own do not ameliorate the biological effects of the snake venom without the action of diverse phytochemicals present in them. Here, we report on the effect of Kihadalactone (a limonoid) 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 sourced from 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.

Reagents

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

Sample Preparation

The purfified kihadalactone sample was obtained after repeated chromatography and characterization. A stock solution of 1 mg /ml kihadalactone was prepared in PBS, pH 7.4. Dilutions of 0.1 mg/ml, 0.01 mg/ml and 0.001 mg/ml of kihadalactone were made from the stock respectively.

Plasma Collection Fresh bovine blood was collected from the abattoire into heparinized tubes and then centrifuged to obtain the plasma.

Determination of Plasma Recalcification Time

The modified method described by Theakson and Reid[5] was used to determine the effect *Naja katiensis* and *Bitis arietans* on plasma recalcification time. Heparinized bovine plasma (100 µl) was incubated in a water bath at 37 ^{o}C. To the sample, 100 µl of crude venom dilution was added; the mixture was then diluted with 100 µl PBS, pH 7.4. Finally, 100 µl of 25 mM $CaCl_2$ was added and the recalcification time recorded using a stop-watch.

The effect of kihadalacetone on the recalcification time on plasma incubated with venom was determined by replacing the PBS above with 0.1, 0.01 and 0.001 mg/ml dilutions of kihadalacetone in PBS. These were conducted in triplicates and the recalcification time was given as mean ± SD.

RESULT

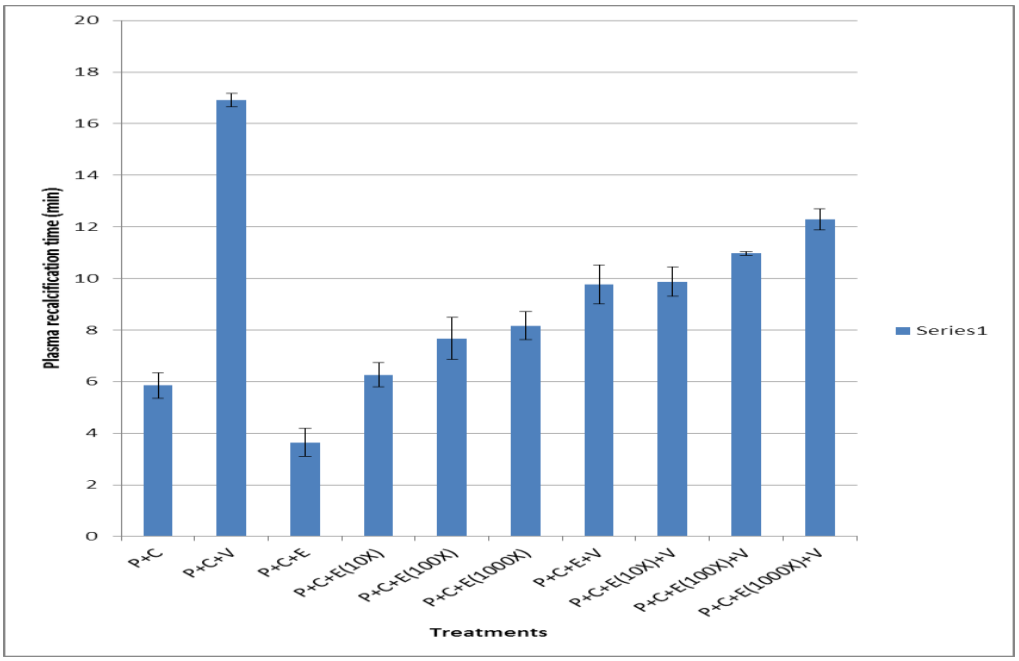

Figure 1: Effect of Kihadalacetone on plasma recalcification time of *N. katiensis* venom

Key: P = plasma; C = $CaCl_2$; PBS = phosphate buffer saline; V = venom (1 g/100ml); E = extract (1 mg/ml); X = dilution

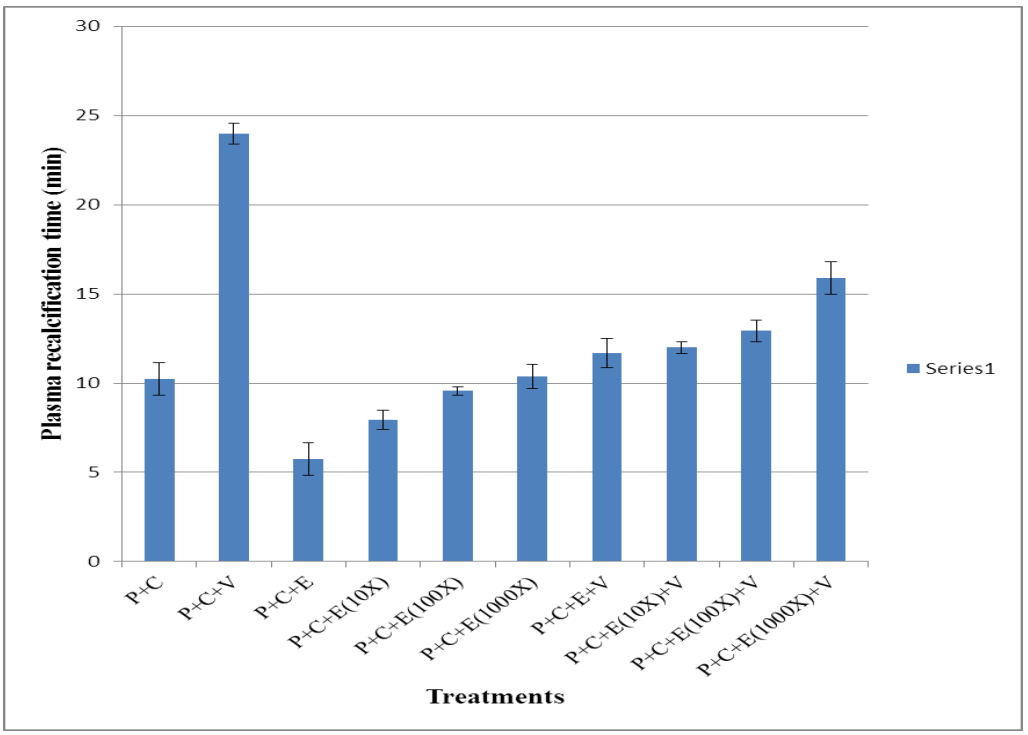

Figure 2: Effect of Kihadalacetone on plasma recalcification time of *B. arietans* venom

Key: P = plasma; C = $CaCl_2$; PBS = phosphate buffer saline; V = venom (1 g/100ml); E = extract (1 mg/ml); X = dilution

The effect of kihadalacetone on the recalcification time on plasma incubated with *Naja katiensis* venom is depicted in figure 1. The recalcification time of plasma treated with venom only was 16.91 ± 0.25 minutes, 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 Kihadalacetone were 9.76 ± 0.76, 9.87 ± 0.57, 10.97 ± 0.07 and 12.29 ± 0.4 minutes respectively.

The effect of Kihadalacetone on the recalcification time on plasma incubated with *Bitis arietans* venom is depicted in figure 2. The recalcification time of plasma treated with venom only was 23.96 ± 0.59 minutes, 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 kihadalacetone were 11.7 ± 0.82, 11.99 ± 0.33, 12.93 ± 0.6 and 15.9 ± 0.9 minutes respectively.

DISCUSSION

Various plant preparations have been used in ameliorating snake bite envenomation. While some are forkloric and local practices that have existed for ages, little have been scientifically validated. This report presents the use of limonoid, kihadalactone in reversing/reducing the increase in plasma recalcification/thrombin time potentiated by *Bitis arietans* and *Naja katiensis* venoms *in vitro*. Giving the results a critical look, a clear increase in plasma recalcification time by both venoms was dose dependently reduced by the presence of kihadalactone in both cases.

The increase in plasma thrombin times by *Bitis* venom is not suprising, taking into account its thrombolytic action and its role in coagulopathy [4] which is usually fascilitated by proteolytic enzymes, fibrinogenase among others that directly interferes with thrombosis. For the *Naja* venom, the presence of PLA_2 have been reported by some *Naja* spp including *Naja nigricollis*.[12] It is not out of place that this same enzyme, which could be present in *Naja katiensis* venom may have caused some disruption in the integrity of the thrombocytes by delocalization and solubilization of some of thrombocyte membrane phospholipids and glycolipids thereby ultimately compromising blood clot formation. Similarly, the likely interference of the enzyme PLA_2 with erythrocyte membrane phospholipids could disrupt its membrane microarchitecture thereby reducing its ability to form the polyhedrocyte, a shape usually formed by erythrocytes during blood clotting and very important in the process.[13] The antagonizing action of kihadalactone with regards to the explained likely biochemical and physiological events that have occurred as a result of the venom have indicated that the limonoid may have had a form of interaction at one or multiple stages in the whole process of

blood clotting that caused inhibition of the obvious biological effect potentiated by the venoms. The π electron rich furan ring present in the kihadalactone may have fascilitated some form of complexation with the snake venom proteins directly involved in the process thereby denying its involvement prothrombin time extension and effectively reversing the effect of the venoms on the clotting process. The result of this may have given an insight into potential application of kihadalactone in managing coagulopathies that directly involve proteins that inhibit clotting factors directly like those of the two snake venoms studied.

CONCLUSION

From the research findings, it could be concluded that the limonoid (kihadalactone) has effectively inhibited the extension of plasma recalcification time *by Bitis arietans* and *Naja katiensis* venoms. The findings are therefore very significant in the development of potent antivenin that are targeted towards ameliorating the biological effect of such venoms or venoms with similar biological activities.

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