



EFFECT OF METHANOLIC STEM-BARK EXTRACT OF *Commiphora pedunculata* ON PLASMA RECALCIFICATION TIME OF *Najani gricollis* VENOM TREATED PLASMA

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ABSTRACT

Several medicinal plants have been used in Nigerian communities as antidotes for snakebite complications without scientific validation. In this study, the effect of the methanolic extract of Commiphora pedunculata on recalcification time of bovine, caprine, ovine and camelid plasma treated with Najani gricollis venom was investigated. The N. nigricollis venom was found to significantly increase (p<0.05) the plasma recalcification time of all the plasma samples. However, the methanolic extract of C. pedunculata at 1, 10 and 100 µg/ml reduced the venom-associated increase in the plasma recalcification time in a dose dependent pattern except for camelid plasma where the effect of the extract was non-dose dependent. This study showed that methanolic extract of C. pedunculata could serve as a prototype for an antidote for the reversal of increase in plasma recalcification time posed by N. nigricollis venom.

Key words: *Commiphora pedunculata*, *Najanigracollis*, *Plasma recalcification time*.

INTRODUCTION

Snake bite envenomation still remains a neglected public health problem in most parts of Africa and is usually associated with impoverished farming populations. It is responsible for about 7,300 deaths and nearly 6,000 amputations resulting from over 314,000 bites that occur annually in Sub-Saharan Africa alone, with one-fifth of all cases occurring in Nigeria (Habib, 2013). Snake venoms are rich natural sources of biologically active molecules which are mostly proteinaceous in nature (Calderon *et al.*, 2014), and capable of affecting physiological processes such as haemostasis, the complement system and neurotransmission (Vyas *et al.*, 2013).

Snake venom haemotoxins affect platelet function and blood coagulation (Koh *et al.*, 2006) which are usually responsible for haemorrhage characterized by consumption of clotting factors and blood incoagulability observed in envenomed patients (Kamiguti *et al.*, 1998). Among the different species of snakes, *N. nigricollis* (black necked spitting cobra) venom has been considered as one of the highly haemotoxins-rich venom which makes it responsible for severe and very fast external haemorrhaging and tissue necrosis around the bite area (Naja, 2008). Antivenoms remain the only specific antidote to snake venom and is capable of reversing all systemic manifestations of envenoming when administered at the right time. However, it is not usually effective against the local haemotoxic effects of *N. nigricollis* venom leading to long term disability and disfigurement. Furthermore, the scarcity and cost of

antivenom coupled with the triple problems of inadequacy and inaccessibility in healthcare had made recourse to the use of local plants with antivenin properties an appealing alternative (Sallau *et al.*, 2015).

Commiphora pedunculata (Kotschy & Peyr.) Engl. belongs to *Burseraceae*, a family composed of both trees and shrubs of tropical and sub-tropical geographical distribution. The plant is used in the folkloric treatment of a number of diseases of public health concern but scientific validation on the therapeutic potentials of the plant has not been conducted for most of the diseases. Studies conducted on the plant mainly focused on antimicrobial activity (Tajuddeen *et al.*, 2014; Tajuddeen *et al.*, 2016). Hence, despite the reported use of *C. pedunculata* for the treatment of snakebite-associated haemorrhage, there is no information on the effect of *C. pedunculata* extract on plasma recalcification time of *Najani gricollis* venom treated plasma.

Plasma recalcification time (PRT), a measure of time taken to clot or recalcify is a physiological process involving the activities of clotting factors, enzymes and calcium ions. A prolonged plasma recalcification time is suggestive of coagulopathy.

This study was therefore conducted to validate the ethnomedicinal claims for the antiophidian effects of the plant by investigating the effects of the methanolic extract on the plasma recalcification time of *Najani gricollis* venom treated plasma.

MATERIALS AND METHODS

Plant Collection and Identification

The stem bark of *C. pedunculata* was collected from Basawa, Zaria, Nigeria, and authenticated at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, where a voucher specimen number 219 was deposited for reference purposes.

Plant Preparation and Extraction

The stem-bark sample of the plant was cleaned, air dried under shade and ground into powder. The dried powder (500 g) was extracted with methanol (2.5 L) in a Soxhlet apparatus for 48 h at 65°C. The entire process was repeated twice. The methanolic extracts were pooled together and filtered using a filter paper (Whatmann size no.1). The filtrate was evaporated to dryness in a water bath at 40°C. The dried extract obtained was weighed and kept in an airtight bottle in a refrigerator at 4°C.

Venom collection and Preparation

Najani gricollis venom was obtained by milking a black-necked spitting cobra (*Najanig ricollis*) maintained at the Serpentarium of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria. The venom was collected with the help of a skilled snake handler. The pooled venom was thereafter placed in a desiccator containing activated silica and allowed to dry at room temperature. The crystallized venom was subsequently transferred into a refrigerator and stored at -18°C.

Plasma collection

Fresh caprine, ovine, bovine and camelid blood samples were collected into heparinized tubes from Kano state abattoir, Fagge Local Government, Kano, and then centrifuged at 3000rpm for five minutes to obtain the respective plasma samples.

Determination of Plasma Recalcification Time

The modified method described by Theakson and Reid (2003) was used to determine the effect of *Najani gricollis* venom on plasma recalcification time. Heparinized plasma sample (100 µl) from each of the animals was incubated in a water bath at 37°C and 100 µl of crude venom dilution (1mg/ml) was added. Thereafter 100 µl of PBS (pH 7.4) and CaCl₂ (25 mM) were sequentially added and the recalcification time recorded using a stopwatch.

The effect of the methanol extract on the recalcification time of plasma incubated with venom was determined by replacing the PBS with 100, 10 and 1 µg/ml dilutions of the extract in PBS.

Statistical Analysis

All data are presented as mean ± standard deviation of three replicate determinations. Data were analyzed using students t-test and values were considered significantly different at $p < 0.05$.

RESULTS

The effect of methanolic extract of *C. pedunculata* on the plasma recalcification time of caprine plasma incubated with *N. nigricollis* venom is presented in figure 1. The recalcification time of caprine plasma treated with venom only was 25.5 ± 0.5 minutes while the recalcification time for incubated plasma treated with 100, 10 and 1 µg/ml of the methanolic extract were 7.5 ± 0.5, 8.5 ± 0.5 and 9.5 ± 1.5 minutes, respectively. The venom was found to prolong the recalcification time of ovine (figure 2), bovine (figure 3) and camelid plasma samples to 21.0 ± 1, 21.5 ± 0.5, 23.5 ± 0.5 minutes respectively. However, the methanol extract reduced the venom-associated increase in the recalcification time in a dose dependent pattern except for camelid plasma where the effect of the extract was non-dose dependent

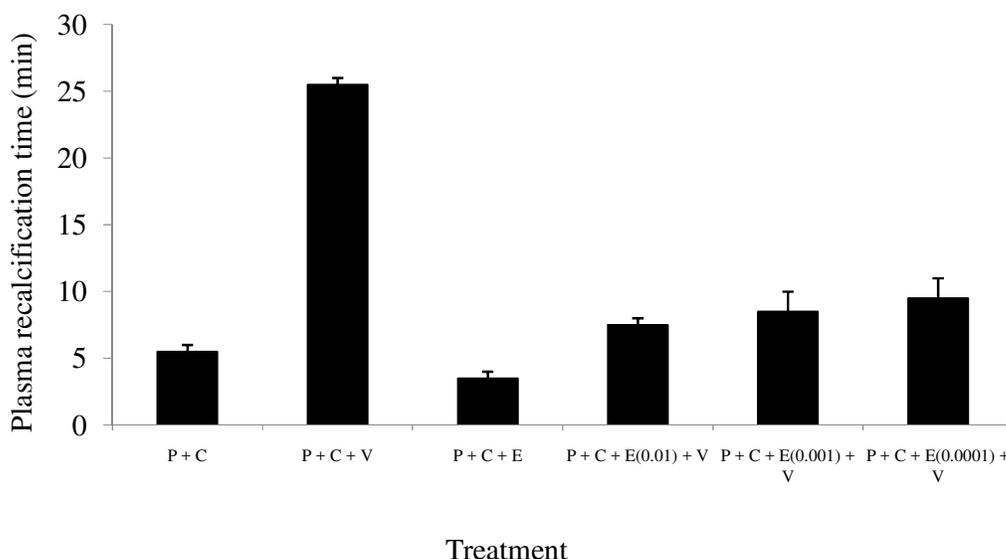


Figure 1: Effect of methanolic extract of *C. pedunculata* on plasma recalcification time of *N. nigricollis* venom treated caprine plasma

P=Plasma; C=CaCl₂; PBS=Phosphate Buffer Saline; V= Venom (1g/100ml); E=Extract (1mg/ml); X=Dilution

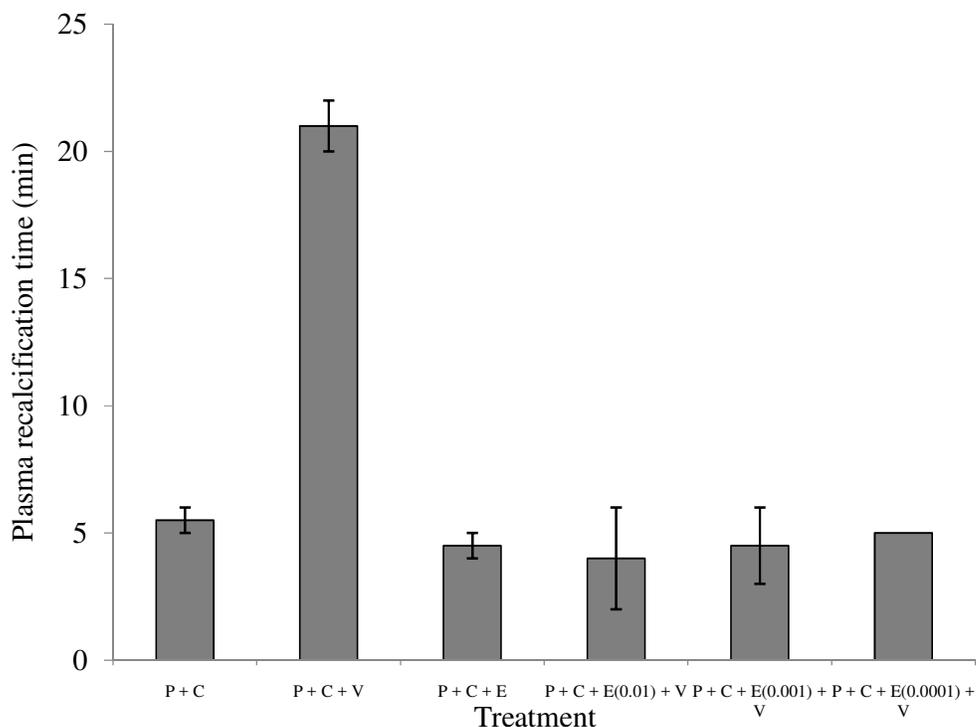


Figure 2: Effect of methanolic extract of *C. pedunculata* on plasma recalcification time of *N. nigricollis* venom treated ovine plasma
 P=Plasma; C=CaCl₂; PBS=Phosphate Buffer Saline; V= Venom (1g/100ml); E=Extract (1mg/ml); X=Dilution

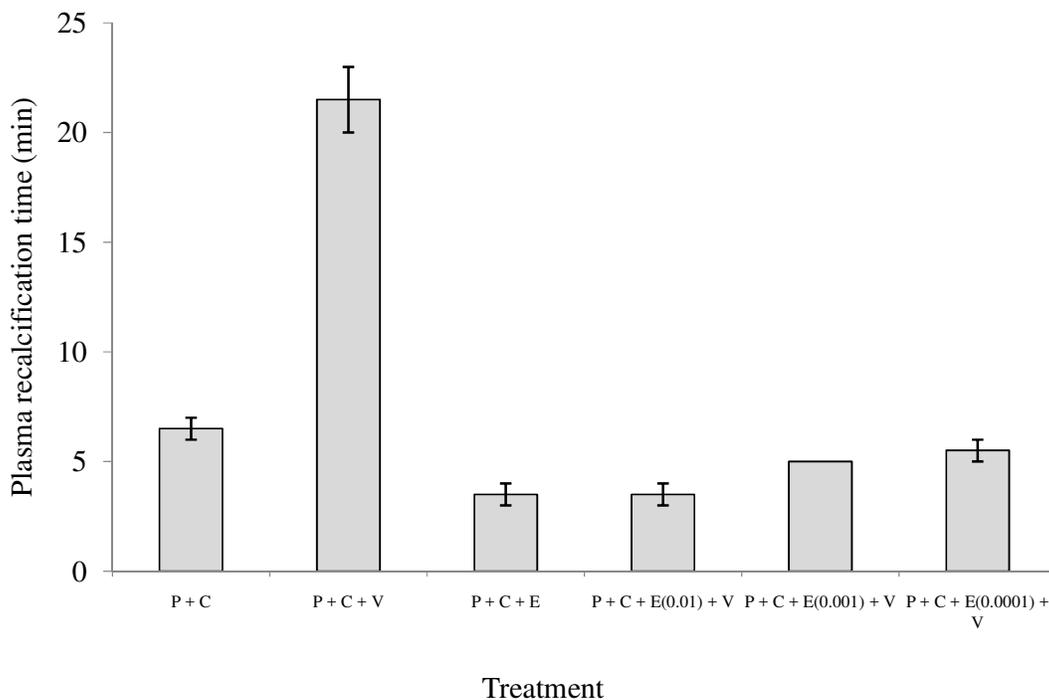


Figure 3: Effect of methanolic extract of *C. pedunculata* on plasma recalcification time of *N. nigricollis* venom treated bovine plasma
 P=Plasma; C=CaCl₂; PBS=Phosphate Buffer Saline; V= Venom (1g/100ml); E=Extract (1mg/ml); X=Dilution

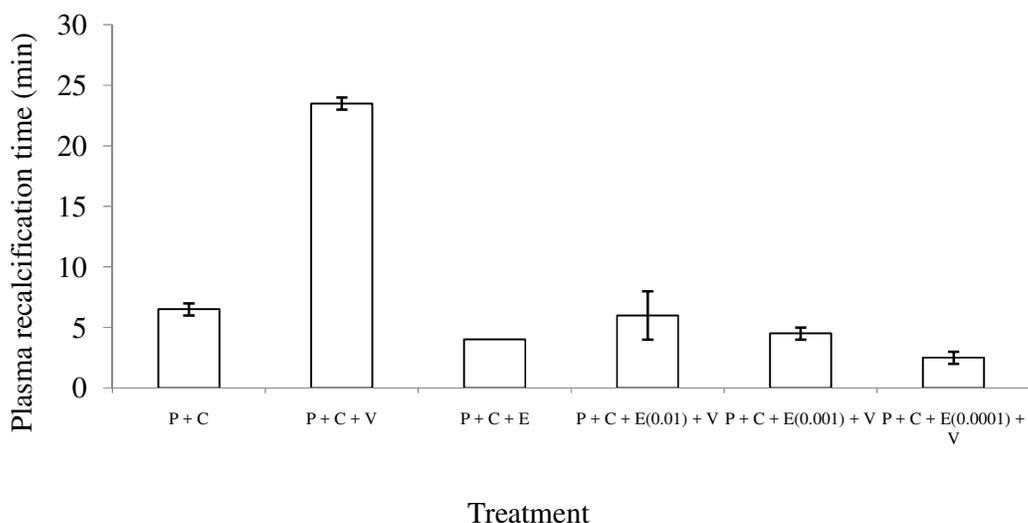


Figure 4: Effect of methanolic extract of *C. pedunculata* on plasma recalcification time of *N. nigricollis* venom treated camelid plasma

P=Plasma; C=CaCl₂; PBS=Phosphate Buffer Saline; V= Venom (1g/100ml); E=Extract (1mg/ml); X=Dilution

DISCUSSION

The role of medicinal plants in the treatment of snakebite has been long recognized. So far, information on the antivenom potentials of *C. pedunculata* has not been documented. In this study, *C. pedunculata* methanol extract was found to profoundly reduce the *N. nigricollis* venom-associated increase in recalcification time of the plasma samples from various animal models.

The highest increase in plasma recalcification time was observed in caprine plasma (Figure 1); that of camelid plasma was slightly lower. This could be attributed to the susceptibility of blood clotting components of these animals to haemotoxic snake venom components. Camelid plasma is rich in clotting factor FVIII:C (Abdel Gader *et al.*, 2013) which serves as abundant substrate for some snake venom haemotoxins following envenomation. This effect occurs rapidly following envenomation and this possibly leads to prolonged plasma recalcification time. This finding is supported by a study conducted by Cook *et al.* (2010), which concluded that a camelid polyspecific IgG antivenom was not effective against the venom-induced effects of *Najani gricollis* venom.

The methanolic extract of *C. pedunculata* affected ovine, bovine and caprine plasma types in a dose-dependent manner (Figures 1, 2, and 3). However, PRT for camelid plasma treated with venom was decreased in a reversed dose-dependent manner (Figure 4); lowest concentration of the extract was most effective against the haemotoxic effect of the venom. This could possibly be due to the presence of a natural slow-acting competitive inhibitor of the snake haemotoxin inherent in camelid plasma which displaces the molecules of the active component of the extract from the haemotoxin's binding site.

The observed reduction of plasma recalcification time by extract indicates that the *C. pedunculata* is endowed with compounds such as tannins, flavonoids

and phytochemicals capable of binding and inhibiting the proteins and enzymes of the clotting cascade via a yet unknown mechanism. A number of phytochemicals such as tannins and flavonoids have been reported as being capable of altering the clotting cascade through binding to the relevant enzymes and proteins (Premendran *et al.*, 2011). Interestingly, flavonoids (kaempferol, dihydrokaempferol and epicatechin) have been isolated and characterized from this methanol extract (Tajuddeen *et al.*, 2014; 2016) and it is thus possible that, these flavonoids along with other phytochemicals mediated the observed effect on plasma recalcification time.

CONCLUSION

The findings of this study clearly confirm that *Najani gricollis* venom is capable of prolonging plasma recalcification time. This effect could however be counteracted by the methanolic extract of *C. pedunculata*, though the extract does not have a neutralizing effect on all venom components.

This clearly lends support to the use of plants for the treatment of snakebite related complications. *C. pedunculata* extract could further be studied as a potential prototype for an antidote against snake venom haemotoxicity.

Author's Contributions: M.A.Ibrahim conceptualized the study; M.A.Ibrahim and H.L. Abdullahi designed the experiments; N.Tajuddeen and S.A.Hamza collected the plant material and prepared the methanol extract; H.S. Muhammed collected all the blood samples and performed the plasma recalcification time experiment together with H.L. Abdullahi, H.S. Muhammed, H.L. Abdullahi, N.Tajuddeen and S.A.Hamza conducted the statistical analysis while H.L. Abdullahi and M.A.Ibrahim drafted the manuscript to the present form.

Conflict of Interest: The authors declare that they have no conflict of interest.

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