Journal of Medical Sciences

ISSN 1682-4474

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original articles in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued four times per year on paper and in electronic format.

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J. Med. Sci., 5 (4): 280-283
October-December, 2005

Effect of *Guiera senegalensis* Leaf Extract on Some *Echis carinatus* Venom Enzymes

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Aqueous extract of *Guiera senegalensis* leaves was investigated for the inhibitory action on the activity of crude phospholipase and metalloprotease enzymes from *Echis carinatus* venom. Both enzymes were inhibited by the extract in a dose dependent fashion. Double reciprocal plots of the initial velocity data of the inhibition by the extract revealed a non-competitive pattern of inhibition for the metalloprotease and a competitive one for the phospholipase. Extrapolated Ki values were found to be 11.9 and 90 μg mL^{-1} for the metalloprotease and phospholipase, respectively.

Key words: *Echis carinatus* venom, *Guiera senegalensis*, metalloprotease, phospholipase

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INTRODUCTION

The *Echis carinatus* belongs to the family *viperidea* and is among the snake species dangerous to humans[1]. The snake is one of the two most common snakes found in northern Nigeria[2] and has been reported by Matsui *et al.*[3] that its venom contains metalloprotease (protease) and phospholipase. These two enzymes are among many enzymes contained in the *E. carinatus* venom found to elicit toxic mechanisms that majorly includes neurotoxicity and mycotoxicity in the case of phospholipase[4,5]. More so, snake venom phospholipase have been noted to have other injurious effects such as heamolysis of red blood cells, anticoagulant action and cardiotoxicity[5]. Snake venom metalloprotease on the other hand is responsible for the severe bleeding observed in snakebites, interference with blood coagulation and haemostatic plug formation or degradation of extracellular matrix components of the victims of the snake bite[3]. These two enzymes therefore, having implicated in such a variety of pathological mechanisms can be said to play central role in the pathology of *E. carinatus* envenomation and possible blockage or inhibition of their action could unveil a way of ameliorating or totally rendering ineffective the toxicity posed by the venom. *Guiera senegalensis* is a tropical shrub of the family *combratacea*[6]. The plant continues to be one of the plants used by local livestock farmers, traditional veterinary healers and Fulani herdsmen in the treatment of snake bite in northern Nigeria. Although Abubakar *et al.*[7] reported reduction in *E. carinatus* venom toxicity by aqueous leaf extract of *G. senegalensis* in their attempt to deduce the likely scientific basis for such practice, the exact target of action of this extract on the venom and the biochemistry involved in such process still remain unknown. There is therefore a need to have an idea of a knowledge of such concept for the effective deduction and use of an active principle from a natural source that is likely very easy to come by with negligible toxic in use in the treatment of *E. carinatus* venom toxicity.

MATERIALS AND METHODS

Snake venom: Freeze dried *Echis carinatus* venom was a gift from Dr. M.S. Abubakar of the Department of Pharmacognosy and Drug Development, ABU-Zaria, Nigeria.

Plant material: Fresh leaves of *G. Senegalensis* was obtained from a bush along Zaria – Funtua road, Zaria-Nigeria and was identified at the herbarium of the Department of Biological Sciences ABU Zaria and has a voucher number of 900141.

Extract preparation: The fresh leaves of *G. senegalensis* were dried at room temperature, 25°C ±2. Ten grams of it was then soaked in distilled water and extracted at 4°C for 36 h. The mixture was then macerated and filtered using cheese cloth to obtain a green coloured suspension which was evaporated to dryness at 50°C to finally get the aqueous extract.

Phospholipase assay: This was carried out by modification of method of Haberman and Neumann[8]. Here, 0.5 mL of egg yolk suspension (2 mg mL^{-1}) was introduced into a clean test tube containing 50 µL of 1 mM $CaCl_2$. To this, 100 µL of 20 mg mL^{-1} venom solution was added and incubated at 37°C for 1 h. Thereafter, the enzymes was inactivated by heating at 100°C for 2 min, drop of phenolpthalin added then titrated against 2 mM NaOH solution to an end point. The same procedure was carried out in the absence of the enzyme in order to obtain titre value for the blank for adequate comparison to deduce effect of the enzyme on the yolk (deduction of any FFA released). The activity of phospholipase was defined as the amount of enzyme required to hydrolyse 1 mg of FFA from the lecithin present in the egg yolk under the standard assay conditions.

Metalloprotease assay: This was carried out by incubating 500 µL of 1 mg mL^{-1} Hammerstein Casein suspension with 25 µL of the venom solution (20 mg mL^{-1}) at 37°C for 30 min. The reaction was stopped by heating the mixture at 100°C for 2 min. Five hundred microliter of ¾ H_2SO_4 and 10% sodium tungstate were added to precipitate the unhydrolysed proteins present which was removed by centrifugation at 9000 g for 10 min. One milliliter of the clear supernatant was collected and heated with 1 mL of Ninhydrin reagent (40%) for 15 min at 100°C to obtain a purple coloured complex for reading at 570 nm Blank was prepared in a similar way with the exception of 25 µL venom solution addition which was replaced by 25 µL acetate buffer pH 5.5.

Effect of aqueous extract of *G. senegalensis* on crude phospholipase: This was carried out using the same procedure as for phospholipase assay only that varying concentrations of the substrate was used and additional 100 µL of 0, 10 and 20% (w/v) of the extract was added to the reaction mixture. The initial velocity data obtained were used for the double reciprocal plots.

Effect of aqueous extract of *G. senegalensis* on crude metalloprotease: This was carried out in a similar fashion as for the metalloprotease assay but 25 µL of varying concentrations of the aqueous extract preparations 0, 5, 10 and 20% were contained in the reaction mixture. The initial velocity data obtained were used for double reciprocal plots.

RESULTS

It is obviously clear from Fig. 1 that the double reciprocal plot shows a non-competitive pattern of inhibition which was dose dependent. Figure 2 presents a double reciprocal plot describing the pattern of inhibition of the extract on phospholipase. The Fig. 2 clearly depicts a classical competitive inhibition pattern which was as well a dose dependent one. Figure 3 and 4 presents Dixon's plots showing how the Ki (Inhibition binding constant) for the extract were estimated for the metalloprotease and phospholipase, respectively. The points of intersection of the plots on the x-axis (representing the concentration of the extract) was used to deduce the Ki, they were found to be 11.9 μg mL^{-1} for metalloprotease and 90 μg mL^{-1} for phospholipase, respectively.

DISCUSSION

The present study represents an attempt made to deduce the exact target of action and the biochemistry of *G. senegalensis* extract detoxification of *E. carinatus* venom which hither to now remained not fully explained.

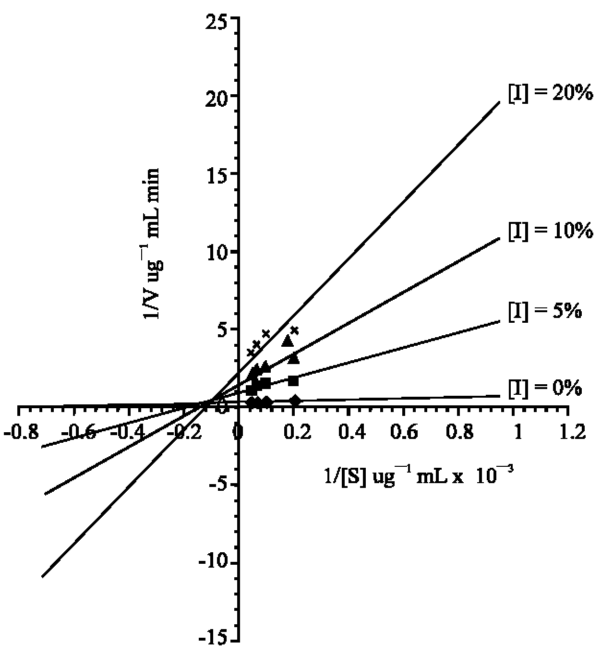

Fig. 1: Effect of aqueous extract of *Guiera senegalensis* leaves on crude metalloprotease activity

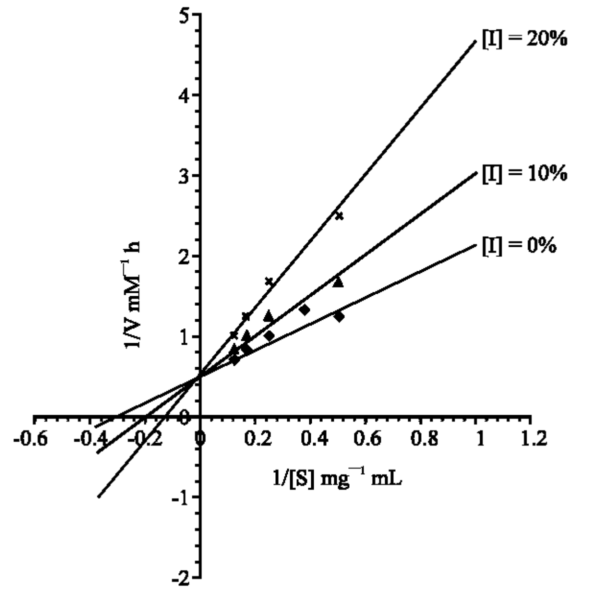

Fig. 2: Effect of aqueous extract of *Geuiera senegalensis* leaves on crude phospholipase

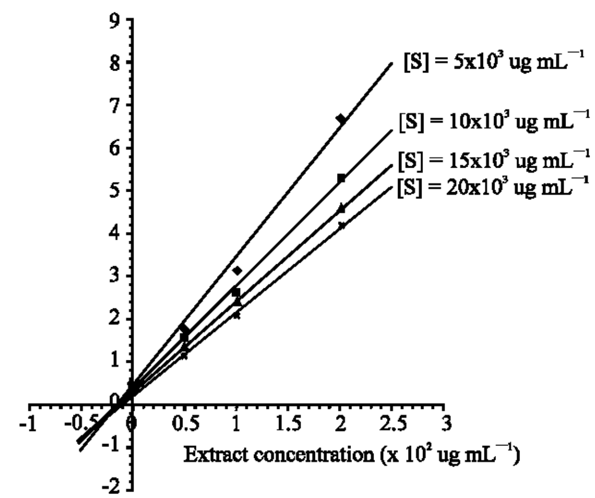

Fig. 3: Dixon's plot to determine inhibition constant (Ki) for the *Guiera senegalensis* leaves extract towards crude metallopratease

In addition this *in vitro* work have further scientifically validated the principle behind the use of *G. senegalensis* plant extract in ethnoveterinary/ethnomedical practices, considering the decreases recorded in both enzymes activities assayed in the presence of the extract. The inhibition of these two *E. carinatus* venom enzymes, taking into account their role in the venom toxicity implies that the extract could serve as a potential candidate for antivenom therapy and could as well be used in the management of *E. carinatus* snake venom poisoning.

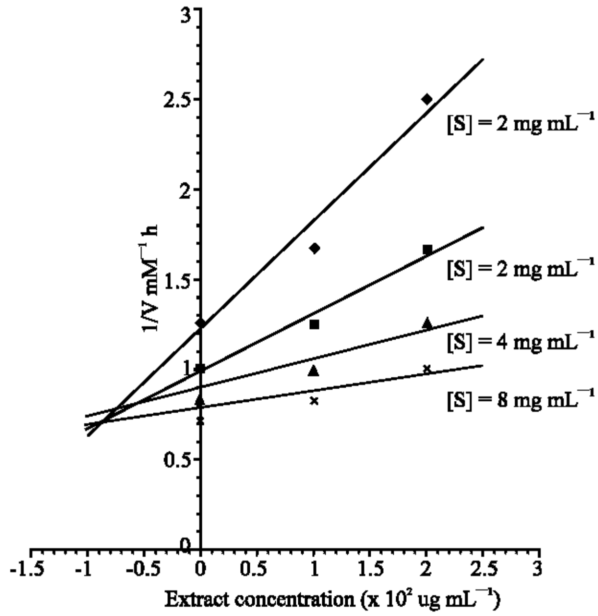

Fig. 4: Dixon's plot to determine inhibition constant (Ki) for the *Guiera senegalensis* leaves extract towards crude phospholipase

The mechanism of inhibition of the metalloprotease by the extract reveal that other sites) on the enzyme apart from the active site could as well be involved in the inhibition although dialysis of the preincubated extract and the enzyme was not carried out to determine whether the inhibition would be reversed. The mechanism also imply that the extract may harbour a constituent that has the capacity to interact with the enzyme substrate complex. The likely constituents of the extract which include polyphenols and tannins are attributable to the reduction in the enzyme activities. Abubakar *et al.*[7] and Okonogi *et al.*[8] implicated tannins in addition to other constituents present in the *G. senegalensis* aqueous extract which are known to unspecifically inactivate proteins to be the likely mechanism involved in detoxifying the snake venom. The pattern of phospholipase inhibition by the extract shows that the active site of the enzyme is involved in the inhibitory action by the extract and more so, that the extract is likely to contain some compounds that could serve as analogues of the phospholipase substrate, by competing for the enzyme's active site. It is as well possible that the extract contains compounds that inactivates the enzyme or interacts with the enzyme rendering its active site unavailable. The dose dependent inhibition of the activities of phospholipase in the venom implies that the proportion of the constituents in the extract could have an effect on the action of both enzymes and that the higher the concentration of the extract the higher the venom enzyme activity inhibition and as such the lower the toxicity ultimately posed by the venom. The low Ki values obtained for the extract towards both enzymes is an indication of a high affinity the extract or its constituent have for these enzymes especially for the metalloprotease which had lower value and therefore is an indication that it could serve as a good source of *E. carinatus* venom antidote and could as well adequately help in designing a novel drug to be used as an antivenin.

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