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# Effect of Some Inhibitors on Clostridium Chauvoei (Jakari Strain) Neuraminidase

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**Abstrac**t: The effect of some inhibitors [silver nitrate ($AgNO_3$), paranitrophenyl oxamic acid (PNPO), Salicyl Oxamic Acid (SOA) and Ethylene Diamine Tetra Acetic acid (EDTA)] on dialysed Clostridium chauvoei (jakari strain) neuraminidase was investigated *in vitro* and it was found that these compounds inhibited neuraminidase activity. The concentrations of the inhibitors used were $1.5x10^3$ μMol, $2.9x10^3$ μMol, $4.4x10^3$ μMol, $5.9x10^3$ μMol and 7.4 μMol for all the inhibitors. Neuraminidase activity was measured by assaying the amount of sialic acid cleaved from fetuin by the enzyme on incubation with the substrate. In all studies, the inhibition was not proportional to the concentration of inhibitor used. It is suggested, based on the present results, that proper clinical trials should be conducted to ascertain the clinical application of these neuraminidase inhibitors in the management of blackleg in cattle.

**Key words:** Inhibitors, clostridium chauvoei (Jakari strain), neuraminidase

## INTRODUCTION

Blackleg is a disease of cattle, sheep and other ruminants caused by Clostridium chauvoei that was first reported in 1870[1]. In Nigeria, the disease was first reported in 1929 by Anon[2] and has remained a major problem of cattle in the country. Although vaccination against it has been carried out since 1930, sporadic outbreaks are recorded annually.

Clostridium chauvoei (jakari Strain), which cause blackleg in indigenous Nigerian cattle, has been found to produce neuraminidase[3]. Neuraminidases (sialidases, EC 3.2.1.18) are involved in the pathogenesis of infectious diseases, whose aetiologic agents produce the enzyme[4,5]. The role of neuraminidase in the pathogenesis of blackleg is that of spreading the disease in host tissues[3]. Neuraminidases are of great importance in medicine and the pharmaceutical industry for the analysis of oligosaccharides and development of neuraminidase inhibitors[6]. The use of neuraminidase inhibitors to manage diseases clinically, especially human influenza virus infections has been advocated[7,8]. As a prerequisite to developing an alternative management protocol against blackleg in cattle, the effect of dialysed Clostridium chauvoei (jakari strain) neuraminidase on fetuin was investigated *in vitro* (both in the presence and absence of neuraminidase inhibitors) and we report here for the first time, the inhibition of Clostridium chauvoei (jakari strain) neuraminidase catalysed hydrolysis of fetuin by silver nitrate ($AgNO_3$), paranitrophenyl oxamic acid (PNPO), Salicyl Oxamic Acid (SOA) and Ethylene Diamine Tetra Acetic acid (EDTA).

## MATERIALS AND METHODS

**Bacterial strain:** Clostridium chauvoei (jakari strain) isolated from clinically infected Zebu cattle was obtained in its lyophilized form from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria for this experiment. The pathogenicity indices of this bacterium have been fully determined[9].

**Bacterial cultivation:** Three media were used for cultivating Clostridium chauvoei (jakari strain) to isolate neuraminidase for this study namely: Reinforced Clostridial Medium (RCM), blood agar and Cooked Meat Medium (CMM). Media preparation and bacterial cultivation were carried out as described previously[10,3] with all the procedures for microbiological asepsis strictly maintained.

Processing of cultivated bacteria: CMM containing the cultivated bacteria was placed in a refrigerated centrifuge (Mistral 4L, MSE) and spun at 9000 g for 40 min at 4°C. The supernatant containing extracellular (crude) neuraminidase released by the bacteria during growth[3]

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was decanted immediately into two sterile plastic containers of one (1) litre capacity each. The plastics containing the culture supernatant were kept at -20°C until when required.

**Ammonium sulphate [$(NH4)_2SO4$] fractionation of crude neuraminidase from clostridium chauvoei (jakari strain):** This was carried out using the method of Useh[3] to determine the fraction with the highest enzyme activity. The highest activity [55-70% $(NH4)_2SO4$ fraction] was pooled and dialysed by modifying the method of Heuermann[11].

**Dialysis of pooled neuraminidase:** Thirty (30) mL of pooled neuraminidase [55-70% $(NH4)_2SO4$ fractionated enzyme] was pipetted into a 20 cm long and 6 cm diameter dialysis bag and placed in a 2 litre capacity dialysis beaker containing 1.5 litres of 50 mM acetate buffer pH 4.5. The dialysis beaker and its content was placed on a speed plate magnetic stirrer at 4°C for 28 h.

**Preparation of 50 mM acetate buffer pH 4.5:** This was carried out using the methods described by Useh[3].

**Preparation of Acid Citrate Dextrose (ACD):** This was prepared using the method previously described[12].

**Preparation of fetuin:** Fetuin in the various concentrations used in the study was prepared using the procedures described else where[3].

**Preparation of inhibitors:** Specified concentrations ($1.5x10^3$ µMol, $2.9x10^3$ µMol, $4.4x10^3$ µMol, $5.9x10^3$ µMol and $7.4x10^3$ µMol) of $AgNO_3$, PNPO, SOA and EDTA, respectively were prepared as previously described[3].

**Hydrolysis of fetuin by dialysed Clostridium chauvoei (jakari strain) neuraminidase:** The hydrolysis (inhibition studies) was conducted as described by Useh[13]. The neuraminidase activity and the degree of enzyme inhibition were quantified by assaying the sialic acid cleaved from fetuin using the thiobarbituric acid assay method[14].

## RESULTS

Hydrolysis of fetuin by dialyzed neuraminidase: The activity of dialysed Clostridium chauvoei (jakari strain) neuraminidase was inhibited *in vitro* by all the compounds tested i.e $AgNO_3$, PNPO, SOA and EDTA. For all the inhibitors used, the rate of enzyme inhibition was not proportional to the concentration of inhibitors used.

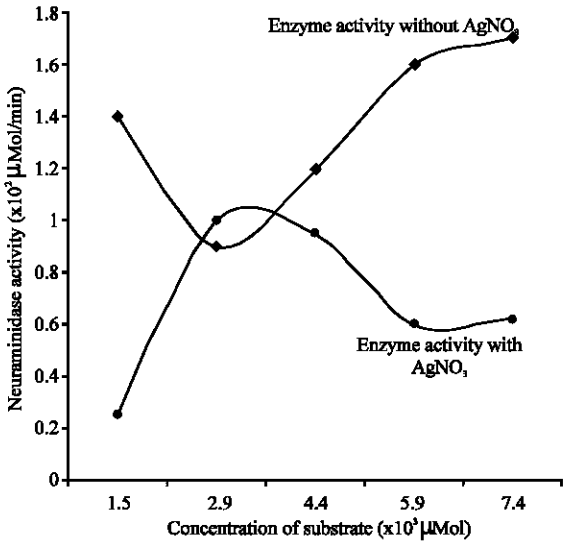


Fig. 1: Effect of silver nitrate ($AgNO_3$) on clostridium chauvoei (jakari strain) neuraminidase

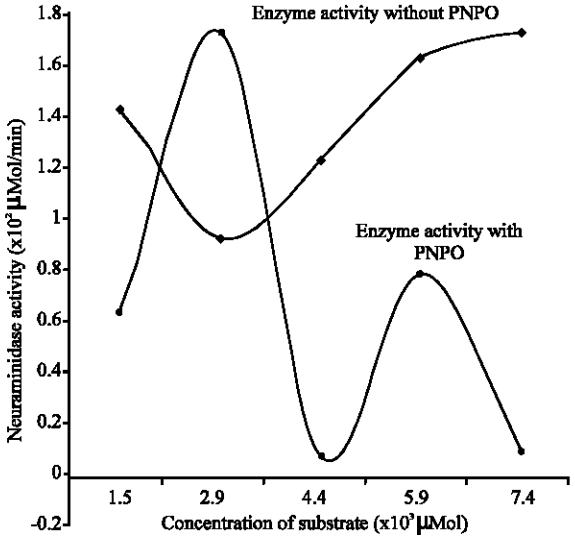


Fig. 2: Effect of paranitrophenyl oxamic acid (PNPO) on clostridium chauvoei (jakari strain) neuraminidase

$AgNO_3$ inhibited neuraminidase activity from $1.4x10^2$ µMol $min^{-1}$ to $2.5x10^1$ µMol $min^{-1}$, $1.2x10^2$ µMol $min^{-1}$ to $9.5x10^1$ µMol $min^{-1}$ and $1.7x10^2$ µMol $min^{-1}$ to $6.2x10^1$ µMol $min^{-1}$ at concentrations of $1.5x10^3$ µMol, $4.4x10^3$ µMol, $5.9x10^3$ µMol and $7.4x10^3$ µMol $AgNO_3$, respectively. There was no inhibition of enzyme activity when $2.9x10^3$ µMol $AgNO_3$ was used Fig. 1.

For PNPO, neuraminidase activity was inhibited from $1.4x10^2$ µMol $min^{-1}$ to $6.0x10^1$ µMol $min^{-1}$, $1.2x10^2$ µMol $min^{-1}$ to 5.0 µMol $min^{-1}$, $1.6x10^2$ µMol $min^{-1}$ to $7.6x10^1$ µMol $min^{-1}$ and $1.7x10^2$ µMol $min^{-1}$ to 7.0 µMol $min^{-1}$

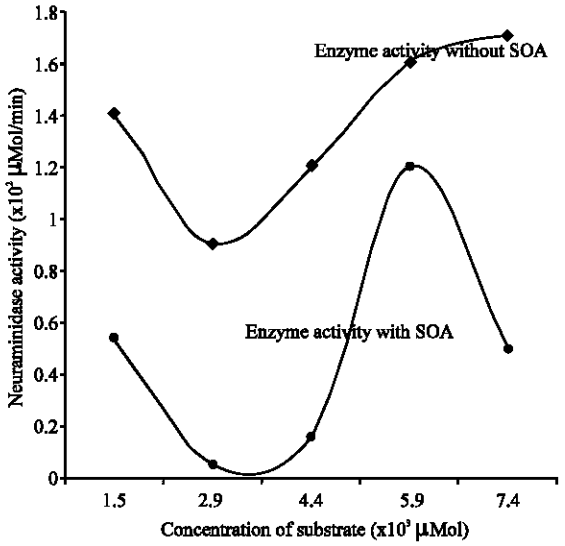


Fig. 3: Effect of salicyl oxamic acid (SOA) on clostridium chauvoei (jakari strain) neuraminidase

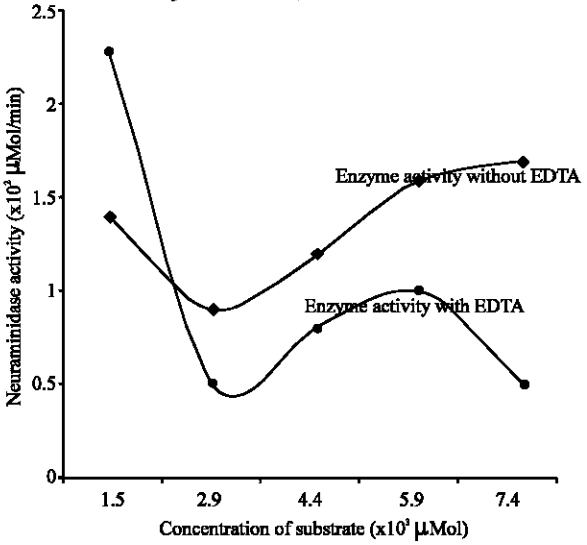


Fig. 4: Effect of ethylene diamine tetraacetic acid (EDTA) on clostridium chauvoei (jakari strain) neuraminidase

SOA inhibited neuraminidase activity from $1.4x10^{2}$ µMol $min^{-1}$ to $5.4x10^{1}$ µMol $min^{-1}$, $9.0x10^{1}$ µMol $min^{-1}$ to 5.0 µMol $min^{-1}$, $1.2x10^{2}$ µMol $min^{-1}$ to $1.6x10^{1}$ µMol $min^{-1}$, $1.6x10^{2}$ µMol $min^{-1}$ to $1.2x10^{2}$ µMol $min^{-1}$ and $1.7x10^{2}$ µMol $min^{-1}$ to $5.0x10^{1}$ µMol $min^{-1}$ when $1.5x10^{3}$ µMol, $2.9x10^{3}$ µMol, $4.4x10^{3}$ µMol, $5.9x10^{3}$ µMol and $7.4x10^{3}$ µMol SOA, respectively were used Fig. 3.

There was also inhibition of enzyme activity when EDTA was used. Neuraminidase activity was inhibited from $9.0x10^{1}$ µMol $min^{-1}$ to $5.0x10^{1}$ µMol $min^{-1}$, $1.2x10^{2}$ µMol $min^{-1}$ to $8.0x10^{1}$ µMol $min^{-1}$, $1.6x10^{2}$ µMol $min^{-1}$ to $1.0x10^{2}$ µMol $min^{-1}$ and $1.7x10^{2}$ µMol $min^{-1}$ to $5.0x10^{1}$ µMol $min^{-1}$ when $2.9x10^{3}$ µMol, $4.4x10^{3}$ µMol, $5.9x10^{3}$ µMol and $7.4x10^{3}$ µMol of EDTA, respectively were used. There was no inhibition of enzyme activity when $1.5x10^{3}$ µMol of EDTA was used Fig. 4.

## DISCUSSION

The terminal sialic acid from sugar residues and glycoproteins is cleaved by the enzyme neuraminidase and the detection of the neuraminidase rests on the assay of free N-acetylneuraminic (sialic) acid split from the substrate[15]. The activity of Clostridium chauvoei (jakari strain) neuraminidase is therefore a measure of the amount of N-acetylneuraminic (sialic) acid cleaved from fetuin.

The result of this study suggests that the compounds used inhibited the activity of dialysed Clostridium chauvoei (jakari strain) neuraminidase *in vitro*. This agrees with the findings of Oladele[16] who also reported a similar finding when these inhibitors were tested on Newcastle disease Kudu 113 virus neuraminidase *in vitro* at different concentrations from those reported in the present study. The inhibition of neuraminidase activity in this study was not proportional to the concentrations of inhibitors used. This disagrees with the former report[16] that the inhibition of Newcastle disease Kudu 113 virus neuraminidase was proportional to the concentrations of inhibitors used. In the present study, there was no inhibition of neuraminidase activity when concentrations of $2.9x10^{3}$ µMol $AgNO_3$, $2.9x10^{3}$ µMol PNPO and $1.5x10^{3}$ µMol EDTA, respectively were used. With this finding, it is tempting to speculate that these concentrations of the compounds that failed to inhibit neuraminidase activity may not be of significance in clinical situations. In the present study, the type of inhibition, whether competitive, non-competitive or uncompetitive was not investigated.

In another study, natural products (Tamarindus indicus and Combretum fragrans) were reported to inhibit neuraminidase activity in a dose dependent fashion and the pattern of inhibition was non competitive[17]. Blackleg is a devastating disease of cattle in Nigeria and annual losses of cattle to the disease in the country have been estimated at six hundred (600) million naira[8]. This is the first time the effect of the compounds used in the present study on Clostridium chauvoei (jakari strain) neuraminidase activity is investigated, in an attempt to circumvent the use of conventional penicillin in the chemotherapy of blackleg. The use of neuraminidase inhibitors to manage diseases clinically has been advocated[18,19]. These inhibitors ($AgNO_3$, PNPO, SOA and EDTA) showed good therapeutic prospect against blackleg *in vitro* and should therefore, be properly investigated *in vivo* to ascertain their efficacy clinically to manage the disease.

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