Full Length Research Paper

Spectrophotometric study of the anti-caseinolytic activity of root extracts of *Teclea nobilis* and *Vepris zambesiaca* on *Bitis arietans* venom

Tigere Chayamiti, Eddie Mwenje and Courtie Mahamadi*

Chemistry Department, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe.

Accepted 15 May, 2013

The study focused on the inhibition of the caseinolytic activity of *Bitis arietans* venom by aqueous methanol crude root extracts of *Teclea nobilis* and *Vepris zambesiaca*. Snake venom samples were collected from Snake World near Harare, and the medicinal plants were obtained from Mash Central Province, Zimbabwe. Data on the kinetics of the digestion of casein by the *B. arietans* venom and the inhibition of the caseinolytic activity of the venom were generated spectrophotometrically. Lineweaver-Burk plots to explore the kinetics of the digestion of casein by *B. arietans* venom gave $V_{\text{max}}$ and $K_M$ values of $8.33 \times 10^3$ mol dm$^{-3}$ s$^{-1}$ and $7.35 \times 10^0$ mol dm$^{-3}$, respectively. Maximum inhibition of caseinolytic activity of the *B. arietans* venom of 70 and 60% was observed when incubation was performed in the presence of 6.25 and 8.50 μg/ml of *T. nobilis* and *V. Zambesiaca*, respectively. The inhibitory effect of the extracts was correlated to the levels of flavonoids, flavonols and phenolics. The results demonstrate that *T. nobilis* and *V. zambesiaca* have great potential as medicinal plants and are possible candidates for new therapeutic agents in the treatment of snake bite envenomations.

Key words: *Bitis arietans*, inhibition, caseinolytic effect, *Teclea nobilis*, *Vepris zambesiaca*.

INTRODUCTION

Snakebite envenomations continue to be a threat to public health in some parts of the world. At least 1,841,000 snakebites resulting in about 94,000 deaths are recorded annually (Chippaux et al., 1991; Pugh and Theakston, 1980). In India alone, 35,000 to 50,000 die of snakebites annually (Morais and Massaldi, 2009). In Zimbabwe, about 270 cases of snakebites are recorded annually with a mortality rate of about 2% (Nhachi and Kasilo, 1994), and a significant number of victims are not reported. Common poisonous snakes in Zimbabwe belong to four families; Colubrids (Boomslang), Vipers (Puff Adder), Elapids (Mambas and Cobras) and Atractaspids (Bibron Stiletto snake) (Muguti et al., 1994).

Snake venom is a complex mixture containing several enzymes, peptides and non-protein components (Chippaux et al., 1991; Rucavado et al., 2004; Pithayanukul et al., 2009). The physiopathological effects of venom results from synergic action of the composite mixture (Currier et al., 2010). The amount of snake venom component depends on the snake species, diet, season and geographical regions (Rodrigues et al., 1998; Currier et al., 2010). *Bitis arietans* venom is mainly haemorrhagic and myotoxic which result from the proteinases and proteases action, mainly metallo-proteinases and serine proteases. Haemorrhagic and proteases activity are the main cause of local tissue damage. *In vitro* assay using casein and fibrinogen are some of the methods used to measure haemorrhagic and proteolytic activities (Biondo et al., 2003). The conventional antivenoms are expensive and do not combat local tissue damage except Batimastat. Although there are several snake venom components,
proteinases mainly cause local tissue damage which has been shown to be of medical importance. Anti-venom immunotherapy has been the only specific treatment against snake envenomations (Arce et al., 2003; Moraes et al., 2003; Gutiérrez et al., 1990). However, there are various side effects of sera antivenoms such as bronchospasms, anaphylactic shock, serum sickness and pyrogen reaction (Lomente et al., 2008; Feofanov et al., 2005; Meenatchisundaram et al., 2009; Wong et al., 2010). Moreover immunotherapy is relatively inefficient to neutral venom-induced local tissue damage (Gutiérrez et al., 2006; Gibbs and Mackessy, 2009), and antivenoms are not always readily available in some regions of Africa and Asia (Chippaux et al., 1998; Rodrigues et al., 1998).

Therefore the search for novel venom inhibitors has expanded to include the possibility of using plants that may neutralize relevant toxins in the venoms and which may be readily available with minimum side effects (Mors et al., 2000; Borges et al., 2001; Biondo et al., 2003; Alam and Gomes, 2003; Januário et al., 2004; Oliveira et al., 2005).

The use of medicinal plants play a significant role to cover the basic health needs not only in the developing countries, but also in developed countries (Selim et al., 2013). Herbs have been a major source of drug formulations, for instance quinine and cough syrups, were developed from plants. Medicinal plants contain many compounds, such as polyphenols (phenolics), flavonoids, flavonols, alkaloids, saponins and steroids which are among the other phytochemical compounds that have been shown to exhibit therapeutic activity, for example antivenom and anti-microbial properties (Mors et al., 2000; Dey and De, 2012; Selim et al., 2013; Barkatullah et al., 2013). The mechanism of action varies from simple chelation of central metal ions, anti-oxidant scavenging to structural modification of the antigens (Mors et al., 2000).

In some regions of Zimbabwe, venomous snakebites have been traditionally treated with infusions of herbs. However, a survey of literature shows that no studies are documented for the inhibitory activities of locally available plants. The aim of this study was to investigate the inhibitory effect of aqueous methanol crude root extracts of two locally available herbs traditionally used in treatment of snakebites, T. nobilis and V. zambesiaca, on the in vitro ability of B. arietans venom to breakdown casein (its caseinolytic effect). Determining the efficacy of extracts from these plants to neutralize the snake venom resources pave way to possible further studies on the development of anti-snake therapy from locally available resources.

**MATERIALS AND METHODS**

The two plants, T. nobilis and V. zambesiaca, were collected in Shamva, Northen Zimbabwe and identified at National Herbarium. The plant roots were dug, cleaned and carried to Bindura University Laboratory in previously cleaned polythene bags.
is the absorbance of casein exposed to venom plus an equal volume of buffer, and $A_{\text{sum}}$ is the absorbance of casein exposed to venom plus an equal volume of sample containing inhibitor (Girish and Kemparaju, 2005). In each case, the test-tubes were incubated at 37°C and scanned after every 1 min using a Shimadzu UV1601 spectrophotometer.

RESULTS

The absorbance of casein shown is shown in Figure 1. Casein was found to be absorbed at 288.5 nm using a Shimadzu UV1601 Spectrophotometer. Caseinolytic activity was examined at this wavelength. Figure 2 shows caseinolytic activity of the venom of *B. arietans*. It can be observed that increasing the venom concentration results in an increase in the rate of caseinolytic activity. The graph helps to determine the minimum venom concentration that brings about a 50% (LD$_{50}$) and 100% (LD$_{100}$) caseinolytic activity. Figures 3 and 4 showed that the kinetics followed a first-order mechanism. Inhibition activity (Figure 5) of the crude plant extracts was correlated to total flavonoid, flavonol and phenolic content in *T. nobilis* and *V. Zambesiaca*, and the linear regression parameters are shown in Table 1.

DISCUSSION

From *in vitro* tests, caseinolytic activity was determined as the ratio of the absorbance of casein relative to the absorbance of the venom-casein mixture. Figures 1 and 2 show the spectrum of casein and the variation of the caseinolytic effect with venom concentration, respectively. The minimum venom dose that resulted in 100% caseinolytic activity was determined to be 0.56 µg/ml. Plots used to obtain kinetic parameters are shown in Figures 3 and 4, and these were obtained using the Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{1}{v_{\text{max}}} + \frac{K_{M}}{v_{\text{max}}} \frac{[S]}{[S]}$$

Where $v$ is the reaction velocity, $K_M$ is the Michaelis–Menten constant, $v_{\text{max}}$ is the maximum reaction velocity, and $[S]$ is the substrate concentration. Values of $K_M$ and $v_{\text{max}}$ were obtained as $8.33 \times 10^{-3}$ mol dm$^{-3}$ s$^{-1}$ and $8.33 \times 10^{6}$ mol dm$^{-3}$, respectively. These results tend to suggest that the digestion of casein by *B. arietans* venom was characterised by fast reaction kinetics.

Caseinolytic activity induced by the crude *B. arietans* venom was inhibited when the venom was incubated with *T. nobilis* and *V. zambesiaca* root extracts at different concentrations (Figure 5). When compared to the inhibitory effect of quercetin as a typical bioactive flavonoid, the plant extracts exhibited a greater inhibitory
effect on the action of the snake venom. Incubating the venom-casein mixture after a dosage of 10.5 µg/ml quercetin resulted in a 50% inhibition, whereas a 70 and 60% inhibition were observed for a 6.25 and 8.50 µg/ml dosage with *T. nobilis* and *V. zambesiaca* extracts, respectively.

The total flavonoid, flavonol and phenolic content of the herbs were higher in *T. nobilis* than in *V. zambesiaca*, as shown in Table 1. These findings tend to suggest that the higher inhibitory effect observed for *T. nobilis* could be explained in terms of the presence of higher levels of bioactive compounds. The mechanisms of action of flavonols, flavonoids and phenolic compounds are through scavenging or chelating process (Girish and Kemparaju, 2005). Such compounds participate in the chelation of the metal atom (zinc) present at the catalytic center of metalloproteinases. Components of the plant extract may occupy sites in the venom, preventing binding of the substrate to the enzymes, and this may take place through covalent or non-covalent bonding (Biondo et al., 2003). However, it suffices to mention that there is still a greater possibility of other bioactive compounds not quantified in this study playing a significant role in the inhibition process.

Substances identified in plants reputed to neutralize the effects of snake venoms, spanning a wide range of molecules, include phenolics, hydroxybenzoic acids, cinnamic acid derivatives, curcuminoids, flavonoids, and tannis, among others (Mors et al., 2000). Additional work to characterise the active phytochemical compounds including those classified under flavonoids, flavonols and phenolics present in *T. nobilis* than in *V. zambesiaca* is
This work was funded by the Research Board... abilities to inhibit the caseinolytic effect of crude... and V. zambesiaca venom under in vitro conditions. A study of some compounds correlated with inhibition of crude venoms showed that the higher inhibitory effect observed for T. nobilis could be related to the higher composition of flavonoids, flavonols and phenolics. This study therefore provides useful initial findings which can be exploited in the continued search for effective therapy on the local effects of snakebites.

**ACKNOWLEDGEMENT**

This work was funded by the Research Board...
through grant number CRP-2/2011.

REFERENCES


